TECHNICAL BRIEF

CLEANER PRODUCTION

Transforming leather production through sustainable innovation

INTRODUCTION

The leather industry is one of the oldest and most economically significant sectors in Pakistan, yet it remains environmentally burdensome, generating significant volumes of effluents laden with harmful heavy metals, organic solvents, and residual chemicals from conventional processing stages. Conventional degreasing, a critical pre-tanning step to remove natural fats and greases from hides, has historically relied on organic solvents and harsh surfactants that contribute substantially to chemical oxygen demand (COD), biological oxygen demand (BOD), and overall effluent quality in tannery wastewater. As environmental regulations tighten and global buyers increasingly demand sustainable supply chains, the leather sector faces mounting pressure to adopt cleaner production strategies that minimize chemical inputs, reduce pollution at source, and improve resource efficiency without compromising product quality. Cleaner production shifts the focus from end-of-pipe waste treatment to the prevention of pollution through the continuous application of integrated environmental strategies across processes and products. In this context, the substitution of chemical-intensive degreasing agents with enzymatic alternatives, specifically microbial lipases, represents a scientifically grounded pathway toward cleaner leather processing, offering the dual advantage of biodegradability and substrate specificity that harsh chemicals cannot provide.

OBJECTIVE

The pilot aimed to produce lipase enzyme in collaboration with Pakistan Council of Scientific and Industrial Research (PCSIR) using solid-state fermentation (SSF). The produced lipase was intended for enzymatic degreasing during leather processing supporting cleaner production in the leather sector by reducing the use of harsh chemicals, reducing water consumption, lowering effluent load, and promoting environmentally friendly processing practices.

METHODOLOGY

1. Microorganism

The lipase enzyme was produced using the filamentous fungus *Aspergillus niger*, which is widely used in industrial fermentation due to its high extracellular enzyme secretion capability.

2. Seed Culture Preparation

Seed culture was prepared using Potato Dextrose Agar (PDA), Yeast Peptone Dextrose (YPD) media , and tributyrin-containing medium to support the growth and activity of *Aspergillus niger*. Fungal spores were inoculated into sterile media and incubated under controlled conditions to obtain an actively growing culture for fermentation. Microbial growth can be represented as:

$$C_6H_{12}O_6 + O_2 \rightarrow CO_2 + H_2O + \text{Microbial Biomass}$$

3. Substrate Bedding

In the next step, the solid substrate was prepared and arranged in fermentation trays to provide a physical support matrix for fungal growth. In solid-state fermentation, the substrate acts as both:

- Nutrient source
- Surface for microbial growth

The bedding step ensures uniform substrate distribution and aeration for fungal metabolism.

4. Hydrating Media Preparation

The hydrating medium was prepared using glucose ($C_6H_{12}O_6$), Potassium Dihydrogen Phosphate (KH_2PO_4), Potassium Chloride (KCl), peptone, and Ferrous Sulfate ($FeSO_4$) as nutrient components to support microbial growth during fermentation. The prepared medium was thoroughly mixed and sterilized by autoclaving for an hour to eliminate any contaminating microorganisms before use.

Dry substrate + Hydrating medium → Moist fermentation substrate

Moisture content is generally maintained at 50-70%, which is optimal for fungal growth.

5. Substrate Tray Preparation

The hydrated substrate was transferred into fermentation trays, ensuring:

- Uniform thickness of substrate layer
- Proper aeration
- Controlled moisture distribution

The trays were then inoculated with the prepared seed culture.

6. Solid-State Fermentation

The inoculated substrate trays were incubated under controlled conditions to allow fungal growth and enzyme secretion.
Typical fermentation conditions are as follows:

- Temperature: 30-37°C
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- Aeration: Natural oxygen diffusion
- Moisture: Controlled hydration

During fermentation, *Aspergillus niger* secretes extracellular lipase enzyme into the substrate matrix.

Organic Substrate → Biomass + Lipase Enzyme

7. Enzyme Extraction

After completion of fermentation, the enzyme was extracted from the fermented substrate using aqueous extraction techniques, where the extraction buffer of Sodium Dihydrogen Phosphate (NaH_2PO_4) and Disodium Phosphate (Na_2HPO_4) was added to solubilize the extracellular enzyme.

Fermented substrate + Buffer → Enzyme Extract

The extract was then separated from solid residues through filtration.

8. Vacuum Drying and Stabilization

The extracted enzyme was converted into a concentrated liquid through vacuum drying to preserve enzymatic activity and extend shelf life. The formulation includes enzyme extract, stabilizing agents and preservatives. Vacuum drying reduces moisture without exposing the enzyme to high temperatures, thereby maintaining its activity.

9. Enzyme Production

The produced enzyme is a vacuum-dried lipase with a brown, thick semi-solid paste appearance. It demonstrates effective activity across a working pH range of 3.0 to 8.0, with an optimum pH of 7.0. The enzyme operates efficiently within a temperature range of 35-45°C, achieving optimal performance at 37°C. In terms of storage stability, it retains over 85% of its residual activity for up to six months when stored at temperatures ≤ 25°C and maintains more than 95% activity for up to twelve months under refrigerated conditions (2-8°C).

APPLICATION PROTOCOL

The enzyme is applied for enzymatic degreasing and lipid hydrolysis in leather processing, with a recommended dosage of 10-20% based on skin weight and a minimum exposure time of three hours to ensure effective degreasing. The lipase catalyzes the hydrolysis of triglycerides into fatty acids and glycerol, which is the fundamental reaction enabling efficient lipid removal. During application, this process leads to the breakdown of triglycerides, formation of emulsified fats, improved removal of lipids, and ultimately enhance dye uniformity in the finished leather.

General reaction:

$$\text{Triglyceride} + \text{Water} \rightarrow \text{Fatty acids} + \text{Glycerol}$$

Chemical equation:

$$C_3H_5(OOCR)_3 + 3H_2O \rightarrow C_3H_5(OH)_3 + 3RCOOH$$

Where:

$C_3H_5(OOCR)_3$ = Triglyceride, $C_3H_5(OH)_3$ = Glycerol, RCOOH = Free fatty acids

RESULTS

Test Results

Skin samples were taken to treat them with pilot enzyme (lipase enzyme), commercial enzyme and kerosene oil to study their degreasing properties and impact on water chemistry. 100 kg pilot enzyme (lipase enzyme) and 8.5 kg commercial enzyme were used to run trials. Tests were conducted in accordance with ISO 4048:2018 for determination of oil and fat content.

Oil & Fat Content Before Degreasing Process			
	Swatch 1	**Swatch 2**	**Swatch 3**
Extracted Matter	10.63%	9.8%	9.84%
Free Fatty Acid Content	5.41%	4.7%	5.40%

Oil & Fat Content After Degreasing Process			
	Swatch 1	**Swatch 2**	**Swatch 3**
Extracted Matter	4.83%	4.85%	4.89%
Free Fatty Acid Content	2.20%	2.41%	2.70%

*Swatch 1 was treated with Pilot Enzyme (Lipase enzyme)
*Swatch 2 was treated with commercial enzyme
*Swatch 3 was treated with kerosene oil

Wastewater discharge was analyzed in accordance with APHA, USEPA, and HACH methods, to check if any changes occur due to chemical inputs (see table below).

Parameters	Unit	Pilot Enzyme (Lipase Enzyme)	Commercial Enzyme	Kerosene Oil
pH@ 25 °C	pH unit	4.07	2.84	3.27
Oil & Grease	mg/l	<5.0	<5.0	21
Total Dissolve Solids (TDS)	mg/l	4712	4040	7392
Total Suspended Solids (TSS)	mg/l	716	604	1166
NH3-N	mg/l	97	128	8.96
BOD	mg/l	435	372	339
COD	mg/l	<5.0	<5.0	21
Total Organic Carbon (TOC)	mg/l	354	249.40	226
NH3-N	mg/l	<1.0	<1.0	<1.0
Total Phosphate	mg/l	<0.04	<0.04	1.53
Surfactant	mg/l	<0.1	<0.1	<0.1
Cadmium (Cd)	mg/l	<0.003	<0.003	0.1
Lead (Pb)	mg/l	<0.005	<0.005	0.5
Arsenic (AS)	mg/l	<0.005	<0.005	1.0
Mercury (Hg)	mg/l	<0.001	<0.001	0.01

KEY FINDINGS

- All three treatments significantly reduced oil and fat content by approximately fifty per cent.
- Both enzymes showed similar efficiency, showing they work equally well in similar working conditions.
- Free fatty acids decreased notably in all samples.
- Lipase enzyme achieved the lowest value (2.20%), indicating better breakdown of fats.
- Wastewater discharge analysis indicated that processing with enzymes generates lower pollution load than kerosene oil.
- Heavy metals concentrations were below detectable limits in both enzymatic methods whereas increased in kerosene oil application.

Environmental Impact

- The application of lipase in leather degreasing supports cleaner production practices by reducing the need for conventional solvent based degreasing agents and minimizing environmental pollution from tannery effluents.
- Enzyme-based processing promotes sustainable leather manufacturing by improving process efficiency, lowering water and chemical consumption, and enhancing the biodegradability of discharged wastewater.

PILOT OUTCOME

The solid-state fermentation process successfully produced lipase enzymes suitable for industrial leather processing applications.

Key outcomes observed during the study include:

i. Successful production of concentrated lipase using *Aspergillus niger*.
ii. Effective hydrolysis of triglycerides into fatty acids and glycerol.
iii. Improved lipid removal efficiency during leather degreasing.

The enzymatic process also demonstrates potential for reducing the use of solvent based degreasing agents, thereby contributing to cleaner leather processing.

CONCLUSION

The study successfully demonstrated the production of lipase enzyme through solid-state fermentation using *Aspergillus niger*. The produced enzyme showed promising potential for application in enzymatic degreasing in leather processing. This biotechnological approach supports sustainable and cleaner production by reducing the use of harsh chemicals, lowering environmental pollution, and promoting more eco-friendly leather manufacturing practices.