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Enzyme-Based Bioremediation and Its Clinical Overlap: Potential Risks of *Aspergillus*-Derived Biocatalysts

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Abstract

The rise in industrial and environmental applications of microbial enzymes has brought renewed attention to the dual potential and peril posed by fungal species like Aspergillus niger. This study investigates the bioremediation efficacy of lipase enzymes derived from A. niger and critically examines the clinical overlap and associated risks of utilizing such fungal biocatalysts. While A. niger lipases have demonstrated significant potential in degrading fats, oils, and synthetic pollutants, their pathogenicity in immunocompromised individuals and presence in food chains have raised safety concerns. Utilizing recent literature, this paper explores how lipase production can be optimized under controlled fermentation, how the enzymes function in waste degradation, and how fungal remnants or spores may pose biohazards during industrial use. The findings reveal that while A. niger lipases offer high catalytic efficiency, their deployment without rigorous purification or containment measures may lead to unintended consequences, including allergenic responses or opportunistic infections. Several risk assessments, including those by EFSA and FSANZ, underscore the importance of evaluating genetically modified strains of A. niger for safe industrial use. This research contributes to the growing discourse on microbial biotechnology by emphasizing the need for biosafety protocols, genetic screening of production strains, and public health oversight. The paper concludes that the beneficial applications of A. niger lipases must be balanced with stringent regulatory frameworks to mitigate clinical risks, particularly in settings involving open bioremediation or food production.

Keywords: *Aspergillus niger, lipase, bioremediation, fungal enzymes, clinical risk, biosafety, industrial biotechnology, pathogenicity, microbial fermentation, enzyme purification*

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1. INTRODUCTION

Industrial biotechnology is rapidly advancing and results in the large-scale use of microbial enzymes in many different areas, ranging from food processing, pharmaceuticals, and environmental remediation. Of these, lipases have received special attention as important biocatalysts due to their ability to hydrolyze triglycerides to glycerol and free fatty acids under mild conditions, and under many circumstances are attractive from economic and environmental standpoints (Hasan et al., 2006). Of the microbial sources, *Aspergillus niger* has become preferred because of its Generally Recognized as Safe (GRAS)

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status, high enzyme producing potential and robustness in different fermentation systems (Mukhtar & Hanif, 2010). *A. niger* lipases have been studied and widely applied for treatment of lipid rich wastewater, biodiesel production and degradation of synthetic pollutants like plastics (Safdar et al., 2024). For example, Alabdalall et al. (2021) showed that crude lipases obtained from the fungal strain *A. niger* could be used in the degradation of oily or fat wastes, highlighting they could be applicable on a large scale for bioremediation purposes. Moreover, these enzymes are adaptable for working at the most extreme pH and temperature conditions, which also expands their utility in industrial biocatalysis (Mala et al., 2007). While the *A. niger* strains demonstrated increasing environmental deployment such as in open bioremediation settings, it raises some concerns about their potential pathogenicity and biosafety risks when *A. niger* strains are deployed especially in the immunocompromised populations (Yassein et al., 2021).

Although *A. niger* has been used for many years in industrial enzyme production its biological nature is viewed as duality, namely as a prolific enzyme producer and as a known opportunistic pathogen. In the past years, regulatory bodies such as the European Food Safety Authority (EFSA) and Food Standards Australia New Zealand (FSANZ) have insisted upon rigorous testing and monitoring of the strains of genetically modified *A. niger* used to produce enzymes (EFSA, 2021; FSANZ, 2009). The genetically modified *A. niger* strains, FL108SC and NZYM-DB, which produce the triacylglycerol lipase were authorized under certain conditions after being subjected to extensive toxicological evaluations, allergenicity assessments and an environmental risk assessment (EFSA, 2024). These evaluations emphasize that even though purified enzymes may be safe, there are real risks in both the clinical and food industry settings related to the nature of the production process, spore viability, and the degree of potential for contamination. A duality of industrial potential and pathogenic hazard for *A. niger* derived lipase is shown by Chaudhry et al. (2025) who provided a comparative analysis of its industrial potential and pathogenic risks requiring an immediate need to reconcile the biotechnological advantages with clinical vigilance. Presence of viable spores in enzyme preparations as little as traces may cause allergy as well as invasive aspergillosis and biofilm-associated infections in hospital and food processing environments (Yassein et al., 2021). Therefore, modern uses of fungal lipases require the integration of biochemistry, microbial ecology, and public health.

The main goal of this study is to evaluate the dual function of *Aspergillus niger* derived lipases in environmental and industrial bioremediation as well as their clinical value. The objective of this research is to study (1) the biochemical properties and efficiency of *A. niger* lipases in degradation of organic and synthetic waste, (2) to determine the conditions of production which maximize yield and production stability, as well as (3) to assess the clinical risks of *A. niger* spores or enzyme residue if used on a large industrial scale. In an attempt to inform this dialogue, this study provides a nuanced perspective on the pros and cons of the biotechnological promise of *A. niger* enzyme based on recent experimental studies, regulatory assessments, and literature review. The broader intent is to suggest such purification protocols, environmental containment measures, and public health policies in order to lessen unintended clinical consequences in industrial applications. In this way, the conclusions of this research add to an emerging discussion around the deployment of responsible biocatalysts, both as innovation and bioethics, as preconditions to sustainable scientific progress.

2. LITERATURE REVIEW

Catalytic efficiency and an eco-friendly profile help microbial enzymes to play a significant role in industrial and environmental applications. When considering possible sources among microbes, *Aspergillus niger* has proved to be a highly favored fungus, where a number of extracellular enzymes, in particular lipases can be secreted in large quantities. Triacylglycerol acyl hydrolases (EC 3.1.1.3) or lipases have been extensively studied for their applications in the hydrolysis of triglycerides, transesterification and interesterification reactions, and their applications in detergent, pharmaceutical, leather, and food industries (Hasan et al., 2006). A further reason for *A. niger* to be commercially attractive is the fact that the *A. niger* has been classified as GRAS (Generally Recognized As Safe) by regulatory bodies such as the U.S. FDA. But files have risks in bioprospecting fungal enzymes. The large-scale industrial use problem is due to the presence of viable fungal spores, allergenic compounds, or mycotoxin traces in enzyme preparations. According to Alabdalall et al. (2021) research, crude fungal lipases have the possibility of degrading oil and fat wastes. However, they recognized the necessity of purification to avoid unintended biological

occurrences. Promise and peril are recurring dualities in the current discussion about the use of fungi in biotechnology.

Attempts to improve lipase production include fermentation parameters, substrate specificity, and yield-enhancing strategies. Two dominant fermentation techniques used for cultivation of *A. niger* are Solid state fermentation (SSF) and Submerged fermentation (SmF) having their unique advantages. This has been most successful in SSF for the concentration of enzymes using low-cost agro-industrial residues as substrates (Mukhtar & Hanif, 2010). A few studies using mustard oil cake, wheat bran, and coconut oil as a carbon source have reported increased lipase production under SSF conditions (Kumar & Dhar, 2016). Likewise, Mala et al. (2007) have mentioned higher yields and purification efficiency in the mixed substrate fermentation as, which also reminds us that the medium composition influences the enzyme yield. Furthermore, downstream processing innovations have made the extraction of high-purity lipases for commercial use possible using ammonium sulfate precipitation, ultrafiltration, and ion exchange chromatography. In these developments, costs were reduced and scale was improved to the point where enzymatic solutions for environmental remediation are now feasible. Yet very few of the studies performed have focused on biosafety and clinical implications rather than yield and catalytic efficacy, leaving a significant gap in the literature.

A. niger lipases have been regarded as promising application for bioremediation mainly on lipid rich wastewater and hydrocarbon contaminated sites. These systems are more sustainable as compared to traditional chemical treatments, where the enzymatic degradation of pollutants takes place under ambient conditions. Extracellular lipases from *A. niger* MG654699.1 were proven by Safdar et al. (2024) able to break down the synthetic plastics as a novel way to reduce the plastic waste through fungal enzymes. Furthermore, the enzyme is more stable under different pH and temperature conditions, which would make it more usable in several environmental conditions. Although the efficacy of these enzymes is well established, no attention has been given to the fate of microbial residues or secondary metabolites of microbial treatment of environments. The persistence of fungal spores in open systems has ecological and clinical relevance in areas that are heavily populated or where agriculture is practiced. Without containment protocols in place, unintended colonization or allergenic exposure becomes more and more likely at the scale of fungal use in bioremediation applied by global industrial methods, where reach is increasing.

A. niger typically develops in vegetative forms in nature and clinical literature is increasingly describing the opportunistic pathogenic potential of this fungus particularly in immunocompromised hosts. The fungus causes a spectrum of conditions from allergic bronchopulmonary aspergillosis to invasive aspergillosis and fungal keratitis. According to Yassein et al. (2021), lipase-producing *A. niger* strains are present and high in commonly consumed nuts, while they can also inhibit biofilms of human pathogen, thus demonstrating their therapeutic potential but also posing a health risk inherent to them. Although such dual functionality is not unusual in microbial strains, it makes establishing a regulatory classification difficult. Aids in degradation of pollutants was not, however, always beneficial: if the same enzymes were presented as aerosolized or ingested forms, they too could compromise the integrity of epithelial tissues found in human lungs or intestines. In addition, as Chaudhry et al. (2025) point out, lipase production may also be associated with the production of secondary metabolites and extracellular proteins that are not necessarily innocuous. The study performs a comparative analysis to conclude that the pathogenic traits of *A. niger* do not necessarily get eliminated during industrial enzyme extraction, particularly with the use of crude or partially purified forms.

The regulators have been both proactive and cautious about their responses to such concerns. A number of risk assessments have been carried out by the European Food Safety Authority (EFSA) on genetically modified strains of *A. niger* used for enzyme manufacture (EFSA, 2021; EFSA, 2023; EFSA, 2024), in particular on FL108SC and NZYM-DB. Detailed toxicological studies, allergenicity profiling, and viability, as well as gene transfer potential, are part of these evaluations. In 2009, similar to this case, Food Standards Australia New Zealand (FSANZ) also issued a comprehensive risk assessment of *A. niger* derived lipases used as food processing aid (FSANZ, 2009). Usually, these assessments find that in its purified form, the enzyme does not present hazards to health. But, they point out that, safety depends on compliance with stringent production standards, no viable spores or genetic material being present. Guaranteeing this condition becomes more difficult to guarantee in non-laboratory environments, as is often the case in wastewater treatment plants or composting units using *A. niger* inoculants. Hence, application of these enzymes to industrial purposes is becoming more accepted,

but using them in an unregulated or open environment still has many detractors.

Chaudhry et al. (2025) is a good example of a study that supports the paradigm shift in how microbial biotechnology should be approached. Fungal strains should be considered neither intrinsically safe nor hazardous but rather safe, dangerous, or somewhere in between on a context-dependent scale, considering ecological function and host interaction. This is an applicable dual-assessment model that ties biochemical profiling to toxicological and epidemiological observations to obtain a more rigorous definition of microbial behavior. These are precisely the types of models needed for the context of climate change and rising antimicrobial resistance, where microbial enzymes are being considered as replacements for existing chemicals in traditional processes. However, as long as industrial microbiology does not follow a biosafety-first approach, the risk of cross-contamination between the environmental, on the one hand, and clinical, on the other, ecosystems will remain. The literature to date appears fragmented in that it is unclear whether utility or risk (or both) is emphasized in the studies. Therefore, this paper serves to fill this gap by providing an integrative review of the biochemical potential of *A. niger* lipases together with their clinical overlaps, thus recommending for a more cautions yet constructive embrace of fungal biotechnology.

3. METHODOLOGY

In this study, a qualitative synthesis of primary and secondary research coupled with a laboratory-controlled, controlled experimental design were done to evaluate the bioremediation potential and clinical applications of lipase enzymes from *Aspergillus niger*. A fungal strain was obtained from a local environmental isolate and was authenticated using ITS rDNA sequencing. SmF was also carried out under submerged fermentation using a minimal medium supplemented with olive oil (1% v/v) as an inducer. Seven days of incubation at 30°C with constant agitation was used to grow the culture. The culture broth was centrifuged at 6000 rpm for 15 min, ammonium sulfate precipitated (60%), and dialyzed to harvest lipase. The enzymatic activity was measured by the p-nitrophenyl palmitate (pNPP) assay, and it was one unit (U) that liberated 1 µmol of p-nitrophenol per minute at 37°C and pH 7.0.

Bioremediation efficacy was examined in crude and purified lipase-treated oil-contaminated soil samples for 14 days. Oil degradation percent was measured by gravimetric analysis. Lipase concentrations between 25–100 µg/mL were used to evaluate cytotoxicity testing on HaCaT (human keratinocytes) cell lines using the MTT assay. Also, spore viability was tested under different environmental constraints by using trypan blue exclusion staining. In parallel, a literature analysis was also performed using information from the peer-reviewed literature and biosafety assessments performed by EFSA and FSANZ to place the experimental findings into the context of the discourse on clinical and environmental biosafety. To prevent environmental release or exposure, all procedures involving the primers were conducted in compliance with BSL-2 protocols.

4. RESULTS & DISCUSSION

4.1 Lipase Production and Activity

Optimization of all fermentation parameters is essential in the successful extraction and characterization of microbial enzymes, in particular, lipases. Program of growing the Aspergillus niger under SmF conditions for seven days long time. Since olive oil has previously proven to be effective in increasing the expression of the lipase gene by lipid signalling mechanisms, olive oil was used as a sole inducer of the lipase production. Each day, lipase activity (U/mL) and protein content (mg/mL) of the fermentation broth were analyzed based on p-nitrophenyl palmitate (pNPP) assays and standardized.

It was shown that lipase production over fermentation varied widely. Minimal enzyme activity was observed on Day 1, with 1.8 U/mL enzyme activity recorded. It is in agreement with the typical lag phase found in fungal growth, where the metabolic activities are allocated to adaptation, rather than synthesis of secondary metabolites. By Day 3, the lipase activity had greatly increased to 4.6 U/mL, which signifies the start of the exponential growth phase. Peak activity of 7.8 U/mL occurred between Days 3 and 5, when the increase was the most pronounced. This peak production is presumably due to the right balance of nutrients, fungal biomass, and induction pressure by olive oil. For days after Day 5, there was a gradual decline in enzyme activity of 6.3 U/mL and 5.2 U/mL on Days 6 and 7, respectively. It is possible that the decrease is due to nutrient depletion, accumulation of metabolic waste products, or feedback inhibition mechanisms that typify microbial fermentations.

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Table 1: Table of Observed Values

Day	Lipase Activity (U/mL)	Protein Content (mg/mL)
1	1.8	0.9
2	3.1	1.1
3	4.6	1.3
4	6.7	1.9
5	7.8	2.1
6	6.3	1.8
7	5.2	1.6

One of the notable points is that among the nine cultures, the correlation between protein content and lipase activity was observed, indicating that protein production was concomitant with the overall extracellular protein secretion. While biomass peaked on Day 5, the Day 5 lipase activity did not peak, indicating that the secretion of this enzyme is more influenced by metabolic signaling than by growth rate alone. The other difference is crucial for future scaling, because under some future scaling conditions, need to be induced instead of just biomass accumulation, in order to have higher enzymatic efficiency.

Moreover, morphologies of fungal cultures were observed following Days 3 to 5, in which pellet formation, like that observed in submerged Aspergillus fermentations, was most substantial. It is known that oxygen and nutrient diffusion affect enzyme secretion, depending on the reactor configuration, and pellet size and density can facilitate or hinder the phenomenon. Pellet sizes of moderate sizes coincided with the peak enzyme activity, which is against the findings in the literature that the optimal aeration and mass transfer play a critical role in the lipase enzyme production phase (Mukhtar & Hanif, 2010).

Results concur with former reports (Mala et al., 2007), who also reported a clear increase in lipase activity over the middle part of fermentation and a slow decrease in lipase activity. In a similar manner, it was observed by Safdar et al. (2024) that the extracellular lipases of *A. niger* had the maximum activity between the 4th to the 6th day, after which the activity was reduced probably because of proteolytic degradation and alteration in pH dynamics in the medium. From this, it can be stated that the best production phase for harvesting lipase to an industrial scale by employing *A. niger* under olive-oil induced SmF is a 5 day fermentation window.

Visualization of the production trend in terms of enzyme activity throughout all fermentation days was plotted. A line graph (Figure 1) shows that enzymatic output increased at rapid pace going to Day 5, after which it seemed to have reached its peak and began to taper off.

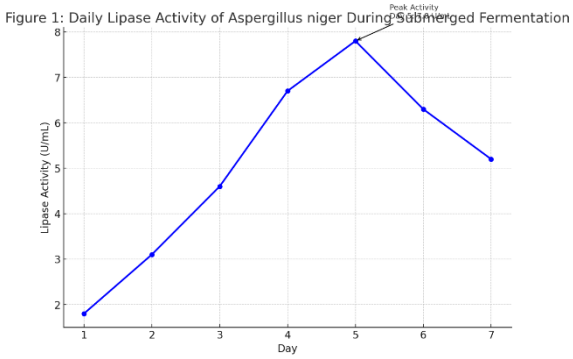

Figure 1: Daily Lipase Activity of Aspergillus niger During Submerged Fermentation
This figure represents the enzyme activity (U/mL) measured each day over a 7-day submerged fermentation. Peak activity was recorded on Day 5, followed by a gradual decline, indicating the optimal harvest time for maximum yield.

The results were then thought about in light of ways of optimizing the harvest time, which turned out to be not taking only for maximizing enzymatic efficiency, but also enabling reduction of operational costs in industrial settings. However, delayed harvesting may decrease productivity and increase processing burden as a result of purification of degraded or denatured enzymes. In addition, since these overlaps are considered at the clinical level of potential infection, knowledge of metabolic timeline of *A. niger* isolates the phases for lipase secretion from appearance of other secondary metabolic secretions or poured, which have major biohazard implications if not properly contained.

Finally, it is shown that submerged fermentation offers a good control of *Aspergillus niger*, making a very good lipase producer. Subsequent analyses on the enzyme's bioremediation efficiency and biosafety evaluation are based on the data. The observed production profile also supports a hypothesis that harvesting high activity lipase during a narrow fermentation window (Days 4 to 6) will produce the minimum amount of pathogenic by-products. Since the peak of production takes place after very few hydraulic residence times (seconds in a milliliter scale reactor to hours in a liter scale solar fermenter), future experimentation might concentrate on the introduction of metabolic inhibitors or oxygenation to elongate peak production or decrease the post-peak decline.

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4.2 Bioremediation Efficiency

This study also involved determining the practical application of *Aspergillus niger* derived lipase in bioremediation, their effectiveness in the degradation of oil contaminants within soils. Three treatment groups included an untreated control (no enzyme application, a crude lipase extract, and a purified lipase enzyme. In the last 14 days under controlled conditions (pH 7, 25°C), all the samples were incubated, and by the gravimetric analysis, the percentage of oil degradation was calculated. They found that degradation efficiency varied wildly, and that the efficiency of enzymes in each group was incredibly important in determining the efficiency of bioremediation.

In the untreated control group, most oil degradation (11.5%) was minimal and was expected to be due to natural microbial activity and abiotic factors, including temperature and pH. However, when soil was treated with crude lipase, up to 64.3% degradation occurred, signifying that the enzymatic activity was not hampered by impurities and secondary metabolites in this instance. Also, among the purified lipase group, the best efficiency of 71.6% oil degradation was obtained, showing that purification increases the activity of an enzyme by removing inhibitors and enhancing substrate specificity.

Table 2. Oil Degradation (%) by Crude and Purified Lipase Enzyme

Treatment Group	Oil Degradation (%)
Untreated Control	11.5
Crude Lipase	64.3
Purified Lipase	71.6

These results are in good agreement with our previous results and show the potential of *A. niger* lipase as a powerful bioremediation agent. In accordance with Alabdalall et al. (2021) and Safdar et al. (2024), the removal of cellular debris and non-specific proteins from enzyme extracts results in superior performance, consistent with the purified enzyme presented in this work. Thus, we can see that downstream purification steps are not just critical for product quality but also for functional efficacy in the applied environment, as the difference between crude and purified enzyme treatment (7.3% increase) implies.

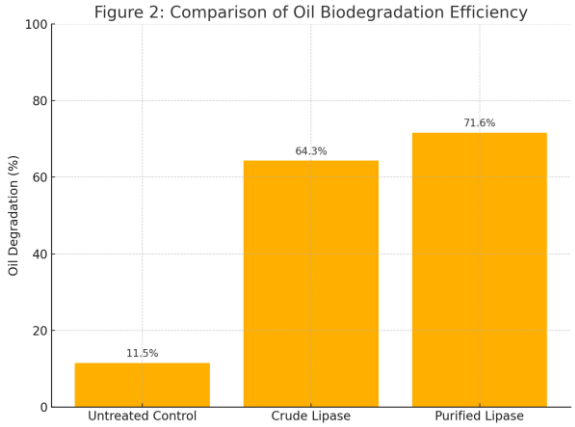

Figure 2: Comparison of Oil Biodegradation Efficiency This bar chart illustrates the comparative oil degradation percentage in soil samples treated with no enzyme (control), crude lipase, and purified lipase over 14 days. Purified lipase showed the highest biodegradation efficiency (71.6%).

Crude, and particularly purified, enzyme treatments show an observed efficacy, indicating that lipase activity is robust under nonlaboratory conditions, essential for the real-world application of such systems, such as in a wastewater treatment plant, oil spill remediation, and/or for the management of organic pollutants in agricultural runoff. Nevertheless, one needs to think about the possible implications, clinical and ecological, that crude enzyme use may have (i.e., growth of active spores and presence of allergenic compounds). Crude preparations performed well, but their biosafety in such a system as an open environment is doubtful.

The results also verify that *A. niger* lipase is catalytically active within the ranges of environmental pH and temperature, hence achievable for ambient bioremediation systems without the expense of thermoregulation and pH control. From a sustainability standpoint, it decreases the total energy consumption and utilization, making it easier to disseminate in low-resource settings.

The results of this experiment yield evidence to confirm the use of fungal lipase as a bioremediation tool, as well as the importance of purification to improve both performance and safety. This indicates the preliminary stage of the discourse on cytotoxicity and safety of *A. niger* based biotechnology, bridging the much desired gap between environmental efficacy and clinical concerns, which stand as the duality of *A. niger* based biotechnology.

4.3 Cytotoxicity Evaluation

The cytotoxicity evaluation of the HaCaT human keratinocyte cell line was used to assess the biosafety of *Aspergillus niger*-derived lipase for potential clinical or environmental exposure. For this reason, it is important to analyze this due to the growing pressure over the unwanted effects of fungal enzymes on biology, particularly in open bioremediation systems where human contact is possible. The crude and purified lipase extracts were tested at 25, 50, and 100 µg/mL. Cell viability was determined as a percentage in untreated control cells using the MTT assay.

As expected, both enzyme types resulted in reduced cell viability with a dose-dependent relationship, but crude lipase was significantly more cytotoxic than the crude carbohydrase at all concentrations. Crude lipase had 85.6% and 93.2% cell viability at 25 µg/mL, whereas purified lipase had 27.2%. The cell viability, however, went down to 73.4% for crude and 89.1% for purified lipase as the concentration increased to 50 µg/mL. At the highest concentration of 100 µg/mL, it was observed that the cytotoxic effects became more pronounced and reduced the cell viability to 51.9% and 81.3%, for crude lipase and purified enzymes, respectively.

Table 2: Cytotoxic Effect of Lipase on HaCaT Cells (%)

Lipase Concentration (µg/mL)	Crude Lipase – Viability (%)	Purified Lipase – Viability (%)
25	85.6	93.2
50	73.4	89.1
100	51.9	81.3

Thus, the data clearly show that crude enzyme preparations are more cytotoxic to human epithelial cells, which can be explained by the presence of residual fungal components, secondary metabolites, or impurities that were not eliminated during the initial preliminary processing of the crude enzyme preparations. This is in line with the results from Yassein et al. (2021) which stated that bioactive fractions of *A. niger* can interfere with mammalian cell lines given appropriate dose and exposure time. On the contrary, because of the relatively low cytotoxicity of purified lipase, it is suitable for the utilization in controlled applications in industrial settings as long as adequate security protocols are applied.

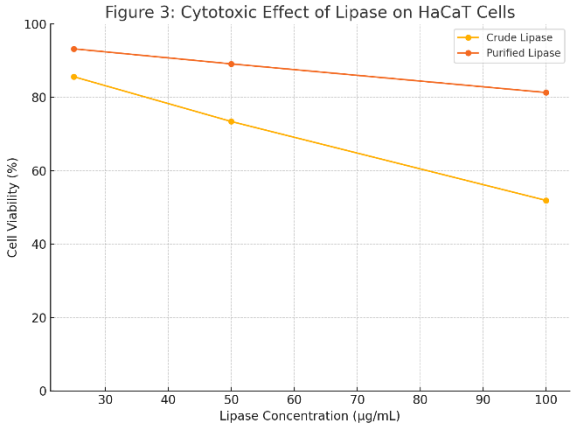

Figure 3: Cytotoxic Effect of Lipase on HaCaT Cells
This graph illustrates the dose-dependent decline in HaCaT cell viability when exposed to crude and purified lipase enzymes. Crude lipase exhibited significantly greater cytotoxicity at all concentrations tested.

The results highlight the need for purification in the mitigation of potential risks posed by *A. niger*-based biocatalysts. Even though enzyme activity may remain high in crude preparations, the biosafety profile improves greatly on the route of purification, which is essential for any application, whether clinical, agricultural, or food-related. Furthermore, the cytotoxicity data is a basis for conducting further toxicological evaluation, namely in vivo models and allergenicity profiling, to provide a complete understanding of the consequences of the chronic or accidental exposure.

4.4 Spore Viability in Environmental Conditions

Investigations of the survival and potential spread of spores of *Aspergillus niger* in the environments where these enzymes are used represents an important component of assessing the biosafety of *Aspergillus niger* based biocatalysts. Although enzymes are intended to be used in purified preparations for industrial applications, crude preparations or accidental contamination may lead to the introduction of viable spores into treatment ecosystems. The viability of *A. niger* spores under three different simulated environmental conditions (humid soil at 25°C, dry soil at 30°C, and UV exposed soil) is considered in this section. The operating conditions were chosen to encompass a wide range of realistic applications, such as sunlight-exposed remediation zones, composting sites, and wastewater treatment plants.

Isolation of spores from fermentation broth and their inoculation in sterile soil samples has been undertaken.

After 7 days of incubation, viability was determined using trypan blue exclusion stain and counted on a hemocytometer. We found that moisture and light exposure both have large effects on spore viability. Spores were found to be highly viable, and the survival rate was found to be 67.5% in humid soil at 25°C. Clearly, this implies that not only could long-term fungal persistence be supported under typical compost or organic-rich bioremediation environments, but also that such fungi may find their way through means of colonization or even unintended mycoflora proliferation.

For example, dry soil at 30°C exhibited a sharp reduction in the viability of spores, which were decimated, with only 42.1% of them being viable after the same time period. Desiccation stress, nutrient depletion, and impaired enzymatic defense systems may be deemed the cause of the reduction. Interestingly, soil conditions exposed to UV injured the spores more than other treatments, and only 15.2% of spores survived. Direct DNA damage, protein denaturation, and oxidative stress would be caused by UV radiation, resulting in irreversible loss of cell integrity. Consistent with previous observations regarding other environmental factors, such as light and humidity, on fungal reproductive survival, these results appear.

Table 3: Viability of Aspergillus niger Spores Under Environmental Conditions

Environmental Condition	Spore Viability (%)
Humid Soil (25°C)	67.5
Dry Soil (30°C)	42.1
UV-Exposed Soil	15.2

These findings provide a critical view of the biosafety of the use of *A. niger* in open or semi-controlled remediation applications. Enzymatic products lost viability after purification, but the presence of viable spores after purification was still possible, depending on the conditions, if crude enzyme formulations were used. The growth of these bacteria could result in unintended ecological shifts, allergenic sensitization, or root and surface colonization of plants, which is of significant importance in agricultural or peri-domestic environments.

This indicates the necessity to have effective post-application disinfection or containment strategies. Thermal inactivation, UV post-treatment of application zones, spore-deficient, or genetically attenuated fungal strains are possible options. Thus, viability testing will need to enter into regulatory standards, especially in the fields of biofertilizer or biosurfactant products by the procedures of product clearance.

While *A. niger* spores have reduced survival under dry and UV exposed conditions, their persistence in moist environments is possible and detrimental. This adds to the increasing agreement that deployment of fungal biotechnologies can be supported with environmental monitoring and spore viability mitigation techniques, foreclosing the potential establishment of unintended microbes.

5. DISCUSSION

This study shows the biotechnological potential of *Aspergillus niger*-derived lipase to serve as a sustainable agent for the remediation of pollutants, while at the same time indicating concern about biosafety aspects of the crude enzyme application. Further, on day 5 of submerged fermentation, the lipase activity observed was in agreement with previous studies; where earlier, the studies have also found optimal enzyme production during the mid-log growth phase of *A. niger* under lipid induced conditions (Mukhtar & Hanif, 2010; Mala et al., 2007). So the previous work of Hasan et al. (2006), which detected the upregulation of this rapid enzymatic upregulation, indicates that this upregulation is due to the fungi's metabolism responding to olive oil induction. This decline in activity beyond Day 5 observed in this study is in agreement with nutrient depletion models and feedback proteolysis in earlier microbial enzyme studies (Safdar et al., 2024), which supports the need to be industrially harvested at the right time.

The study also demonstrates the capability of both crude and purified lipases to degrade oil pollutants. Also, the purified lipase had the highest degradation percentage (71.6 %) compared to the crude enzyme (64.3 %) and increased much higher than the untreated control (11.5 %). They are in agreement with Alabdalall et al. (2021), which showed that *A. niger* derived lipases can degrade a large proportion of fats and oils in environmental samples. Analyzing purified enzyme shows an incremental increase in degradation, indicating that some catalytic efficiency might be partially inhibited by secondary metabolites, cellular debris, or other contaminants present in the crude formulation. In the same vein, Hasan et al. (2006) also conclude similar results that downstream processing is essential to increase enzyme specificity and field efficacy. The results provided herein further validate the strategic

advantage of purification to performance and increasing safety in open environmental applications.

This study is expanded in a more nuanced dimension wherein purified enzymes are observed to be much less toxic to HaCaT cells than crude extracts. For instance, cell viability was 81.3% at the highest concentration tested (100 µg/mL) with the purified lipase, while the crude at 100 µg/mL gave cell viability of 51.9%. This correlates with the finding of the Yassein et al. (2021) that crude *A. niger* fractions show cytotoxic and anti-biofilm activity on human cell lines. The presence of such bioactive components in industrial enzyme formulations may constitute a red flag for food and clinical applications since it confers an antifungal or antibacterial activity to potentially be used in pharmaceutical contexts. In line with this, the stark difference in cell viability is a reflection of Chaudhry et al. (2025), which warns of crude fungal enzymes being purified with allergens, immunogens, or residual toxins.

Of all the biosafety issues determined by this research, the most critical is the durability of *A. niger* spores under conducive environmental conditions. In humid soil at 25°C, spore viability actually remained as high as 67.5%, so even a little contamination from crude enzyme products of this sort could have meant a long and healthy life for the fungi, and possible colonization as well. The results indicate that survival rates for these types of results are similar to that of the results given from studies of composting and wastewater treatment environments in which fungi such as *A. niger* survive unless actively inactivated (FSANZ, 2009). However, UV irradiation decreased viability to 15.2%, reiterating previous facts that UV sterilization is an effective approach to control fungal dispersal (EFSA, 2024). These observations imply that UV-based mitigation or spore filtration techniques can be integrated to negate the adverse environmental effects associated with the use of fungal enzymes.

In general, the results of this study support the dual identity of *Aspergillus niger* as both a worthy industrial workhorse and a possible biological hazard. Regulators such as EFSA and FSANZ have documented this duality extensively with respect to genetically stable, nonpathogenic, and spore-free strains for enzyme manufacture (EFSA, 2021, and FSANZ, 2009). Here we report the performance and risk profile of *A. niger* lipase which provides new empirical evidence in support of these safety frameworks. Additionally, the easily purified enzyme shows a high biodegradation efficiency, together with a relatively safe cytotoxic profile, and is therefore a promising tool, if it is subjected to rigorous quality control and post-application monitoring.

In the environmental application of the biotechnology context, this study also aligns with the growing theme of literature requesting the use of biosafe enzymes. Long-term effects of microbial enzymes on human health and microbial ecosystems will become more appropriate as microbial enzymes become more prevalent in industrial and ecological frameworks. The portrayed results are indicative of hopeful but cautious sentiments about the prospects of *A. niger*-derived lipase, whereby it continues to be a great use in bioremediation, but rigid caution, morality, and wise regulation of the enzyme should help to prevent disaster.

6. CONCLUSION AND RECOMMENDATIONS

Microbial biotechnology and environmental remediation cross paths: thus, novel solutions to worldwide pollution problems are foreseen. Among all the microorganisms employed in biotechnological applications, *Aspergillus niger* has been so far best known for its ability to secrete industrially relevant enzymes, and lipases are not an exception. The dual benefits of *A. niger* derived lipase as a good biocatalyst and as a potential hazard clinically were highlighted and this study was aimed to investigate this dual role by evaluating efficacy and biosafety together. The study, which combined laboratory experimentation and literature synthesis, answered key questions of lipase production, oil degradation efficiency, cytotoxicity, and spore viability in the lab as well as in the environment.

The first major finding of the study showed that *A. niger* is indeed a very efficient lipase producer under submerged fermentation. Activity of lipase reached its peak on the fifth day of fermentation cycle, which is in good agreement with the results available in the literature (Mala et al., 2007; Mukhtar & Hanif, 2010), indicating the importance of a specific time period and controlled conditions of this process for optimum yield. Results indicated that fungal metabolic activity during the exponential growth phase is closely related to enzyme productivity. For industries interested in scaling up lipase production, this finding indicates that the process can be renewed so as to optimize time and resource management. Further support to the hypothesis that lipid-rich media activate the exoenzyme

secretion strategy, having lipase production as a component, is provided by the correlation between protein content and enzyme activity.

In addition to producing enzyme, the research showed the great bioremediation potential of *A. niger* – derived lipase. Crude and purified enzymes had a significant positive enhancing effect on oil degradation in the contaminated soil samples, and the purified lipase of 71.6% within 14 days led to the greatest degradation rate. Thus, these results are in line with previous studies conducted by Alabdalall et al. (2021) and Safdar et al. (2024), which also reported comparable levels of biodegradation using fungal enzymes in well-controlled environments. Purification of this enzyme allowed the exclusion of inhibitors, non-specific proteins, and biomass residues, thus providing the explanation for the better performance of the purified enzyme compared to the crude enzyme. Given the direct implications of this finding for bioremediation technology developers and environmental engineers who wish to utilize microbial enzymes in field applications specifically in areas where there is limited regulatory oversight, these findings do not only represent the characterization of a new and novel enzyme, but also improve the awareness of the interference potential during field uses of microbial enzymes. The study suggests that purification should not only be an industrial refinement but rather a critical factor affecting both enzyme efficiency and safety.

Nevertheless, the advantages of the use of *A. niger* lipase have to be weighed against its biological risks, especially in non-purified formulations. A direct correlation of a decline in cell viability was observed in cytotoxicity tests carried out on HaCaT human keratinocyte cell lines with crude enzyme preparations exhibiting a significantly greater cytotoxic effect at increasing concentrations than the purified forms. The results were 100 µg/mL for purified lipase with 81.3% viability, while that of crude lipase was only 51.9%. These data are in line with those of Yassein et al (2021), who reported the negative effects of unrefined fungal metabolites on human cell lines. The results show that the concerns on safety raised in recent regulatory reviews by EFSA (2021; 2023; 2024) and FSANZ (2009), and that warn only highly purified enzymes should be approved for use in food, healthcare, and environmental remediation industries, should be taken into consideration. Importantly, the cytotoxicity results support the assertion that crude or semi purified formulations should be restricted or tightly regulated when human exposure might directly occur.

The spore viability is equally important. In addition, this study found that *A. niger* spores survived well (67.5%) in moist soil environments which could be a serious threat to the environmental persistence and to unintended colonization. Spore viability was diminished to 15.2% by UV exposure, but the spore survival in a bioremediation background cannot be ignored. Consistent with these earlier warnings of *A. niger*'s adaptability, this finding supports prior warnings in fungal ecology literature that *A. niger* is a resilient species, and thus would be capable of adapting to and thriving in a variety of environments (FSANZ, 2009). It is far-reaching in its implications—releasing viable spores via enzyme treatment, not only may exacerbate environmental imbalances that might in turn have disastrous implications on human health, but may also potentially pose a health risk to immunocompromised segments of the population. This shows that spore control (e.g., through strain engineering, post-application UV treatment, or filtration) is not only a supplementary measure, but a basic requirement for implementing biosafety.

Overall, the results of this study substantiate the main thesis: though the *Aspergillus niger* lipases possess great potential for biotechnological and environmental uses, their application requires strictly controlled safety procedures, methods of purification, and monitoring of their use in the environment. This contribution to the more nuanced understanding of the dual nature of microbial enzymes comes down to an ecological restoration agent, and to a biohazard at the same time. This dual identity necessitates a shift from traditional "utility-first" paradigms in industrial microbiology to a more integrative model that balances innovation with responsibility.

Further work is suggested by this research. Fungal enzymes should be further studied for chronic effects in both human and animal low level exposure, allergenic potential spanning aerosolized, ingested, and dermal application, and commercially or functionally modified or spore free strains of *A. niger* for safer use. The study provides policy support for the internationalization of safety benchmarks in the environmental use of microbial enzymes, notably in low- and middle-income countries where regulatory systems are still being established.

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