MICROBIAL ENZYME PRODUCTION: A PROMISING PATH TOWARDS SUSTAINABLE INDUSTRIES

# **INTRODUCTION:**

In recent years, the concept of sustainability has gained significant attention across various industries. As the world faces pressing environmental challenges and increasing demands for eco-friendly solutions, the need for sustainable practices in industrial processes has become more crucial than ever. One promising avenue towards achieving sustainability lies in the production and utilization of microbial enzymes. These powerful catalysts offer tremendous potential to transform industries by enabling greener and more efficient processes.

The WHO states that industrial activities significantly contribute to environmental pollution, including resource depletion, air and water contamination, and the production of hazardous waste. It is evident that traditional industrial processes relying on chemical-based methodologies have become increasingly unsustainable. Fortunately, microbial enzymes provide a viable substitute that is in keeping with the objectives set forth by the WHO. Microorganisms like bacteria and fungus have a staggering variety of enzymes with amazing catalytic properties. By adopting microbial fermentation processes, these enzymes may be generated in vast quantities and offer an environmentally responsible answer to a number of industrial problems.

Pakistan, like many other countries, faces significant environmental challenges and the need for sustainable solutions across various industries. Embracing sustainable practices is vital to mitigate environmental degradation and promote economic growth while ensuring the well-being of its population. In line with the global movement towards sustainability, the production and utilization of microbial enzymes offer a promising avenue for Pakistan's industries to transition towards greener and more efficient processes.

# **Major Problem: Environmental Degradation and Unsustainable Industrial Practices**

The world is currently facing a major problem of environmental degradation due to unsustainable industrial practices. Chemical-based industries, which heavily rely on non-renewable resources, contribute to pollution, resource depletion, and climate change. If this problem is not resolved, several significant consequences can arise:

* Continued Environmental Pollution: Without transitioning to sustainable practices, chemical-based industries will continue to emit pollutants into the air, water, and soil. This will worsen air quality, leading to respiratory illnesses, cardiovascular diseases, and premature deaths. Water pollution will persist, harming aquatic ecosystems and endangering access to safe drinking water. Soil contamination will impair agricultural productivity and pose risks to food safety.
* Resource Depletion and Scarce Availability: Non-renewable resources used in chemical-based industries, such as fossil fuels and minerals, will continue to be depleted. As a result, these resources will become scarcer and more expensive, potentially leading to economic instability and geopolitical tensions. Dependence on finite resources without exploring sustainable alternatives will hinder long-term industrial growth and development.
* Escalating Waste Generation and Disposal Challenges: Failure to address unsustainable industrial practices will perpetuate the generation of vast amounts of waste, including hazardous and non-biodegradable materials. Improper waste management will lead to further pollution, soil degradation, and risks to human health. The accumulation of waste will strain existing landfill capacities and put pressure on ecosystems, exacerbating environmental challenges.
* Occupational Health and Safety Risks: Workers in chemical-based industries will continue to face occupational health and safety risks associated with exposure to hazardous substances. Without implementing sustainable practices, incidents, accidents, and occupational diseases will persist, jeopardizing workers' well-being. The health and safety of workers will be compromised, resulting in reduced productivity, increased healthcare costs, and social burdens.
* Intensifying Climate Change Impacts: The greenhouse gas emissions produced by chemical-based industries contribute to climate change, leading to severe consequences. Failure to mitigate these emissions will result in rising global temperatures, altered weather patterns, increased frequency of extreme weather events, and sea-level rise. These changes will adversely affect ecosystems, agriculture, water resources, and human settlements, leading to widespread socio-economic disruptions.

Failing to address these challenges will perpetuate environmental degradation, hinder socio-economic progress, and compromise the well-being of present and future generations.

# **REPLACING CHEMICALS WITH MICROBIAL ENZYMES**

Replacing chemicals with microbial enzymes offers numerous benefits in terms of sustainability and environmental impact. Here are some key advantages of using microbial enzymes as replacements:

* Eco-Friendly and Reduced Pollution: Microbial enzymes are derived from natural sources and are biodegradable, making them environmentally friendly. By replacing harsh chemicals with enzymes, industries can significantly reduce pollution levels in air, water, and soil. Enzymes promote cleaner production processes by minimizing the release of toxic substances and harmful byproducts.
* Improved Energy Efficiency: Microbial enzymes typically operate under milder conditions of temperature and pH compared to traditional chemical processes. This reduces energy consumption during production, making the overall process more energy-efficient. Lower energy requirements contribute to reduced greenhouse gas emissions and help mitigate climate change.
* Selectivity and Specificity: Microbial enzymes exhibit high selectivity and specificity, enabling them to target specific reactions with precision. This specificity minimizes the formation of unwanted byproducts, reducing waste generation and optimizing resource utilization. Enzymes allow for more controlled and efficient processes, leading to higher product yields.
* Mild Reaction Conditions: Enzymes often work under mild reaction conditions, which can eliminate the need for harsh solvents, high temperatures, and pressure. This eliminates potential safety hazards for workers and reduces the risk of accidents or chemical-related injuries. Safer working environments improve occupational health and safety standards.
* Reduced Water Consumption: Microbial enzymes can facilitate water conservation by reducing the need for extensive water usage in industrial processes. Compared to chemical-based methods, enzyme-based processes often require lower volumes of water for cleaning, rinsing, and reaction purposes. This helps address water scarcity concerns and conserves this precious resource.
* Renewable and Sustainable Resources: Microbial enzymes can be produced using renewable resources as feedstocks. Biomass, agricultural waste, or byproducts can serve as sources for microbial enzyme production, reducing reliance on non-renewable resources. Utilizing renewable resources aligns with sustainable principles and helps preserve natural resources for future generations.
* Versatility and Multiple Applications: Microbial enzymes have a wide range of applications across various industries, including textiles, food and beverages, detergents, pharmaceuticals, biofuels, and more.

# **SELECTED MICROBIAL ENZYME FOR REPLACEMENT**

The enzyme we have selected to start replacing chemicals in various industries is “PROTEASE”. Here are some examples of how protease can be utilized in different sectors:

* Textile Industry: Protease enzymes are widely used in the textile industry for processes such as bio finishing and bio-scouring. They can help remove unwanted substances from textile fibers, such as natural impurities or chemical residues, resulting in cleaner and smoother fabrics
* Food and Beverage Industry: Protease enzymes play a crucial role in food processing. They are used to enhance the flavor, texture, and nutritional value of various food products. Proteases can be utilized in the production of cheese, where they aid in the ripening process and contribute to flavor development. They are also employed in the brewing industry to break down proteins and prevent haze formation in beer, leading to improved clarity.
* Detergent Industry: Protease enzymes are commonly included in laundry detergents. They help break down protein-based stains such as blood, grass, and food residues, thereby improving the effectiveness of stain removal. Proteases in detergents enable more efficient cleaning, even at lower temperatures, which reduces energy consumption and contributes to environmental sustainability.
* Pharmaceutical Industry: Protease enzymes find applications in the pharmaceutical field, particularly in the production of medications. They can be used in the synthesis of peptides and proteins, aiding in the development of therapeutic drugs. Proteases are crucial in protein engineering and can be employed to modify and optimize the activity of proteins and peptides for pharmaceutical applications.
* Environmental Industry: Protease enzymes can be utilized in wastewater treatment processes to degrade organic matter. They help break down proteins present in wastewater, aiding in the removal of pollutants and improving the efficiency of the treatment process. By using proteases, industries can contribute to cleaner water systems and reduce the environmental impact of wastewater discharge.

# **MATERIAL AND METHODS**

## UPSTREAMING

### ISOLATION OF MICRORGANISM

### The soil samples will be taken from different gardens were inoculated in nutrient broth.

### The cultures will be diluted with physiological saline water and transferred into nutrient agar through pour plate or spread plate techniques.

* Then gram staining will be done to identify gram positive bacilli shaped bacteria.
* Then selected colonies will be purified using streak plate method and then confirmed its purity by gram staining.
* Then it will be sub-cultured at regular intervals to maintain viability.

### SCREENING FOR ACTIVITY

### Qualitative

Protease activity will be measured by hydrolysis of casein (1% w/v) pH-8 at 37°C. Single colony was transferred into skim milk agar for screening protease activity.

Quantitative

Enzymatic assay using **McDonald and Chen method will be done for the quantitative measurement of protease being produced.**

### STRAIN IMPROVEMENT THROUGH DIFFERENT MEDIUM COMPOSITION

### Screened positive bacteria are inoculated in 5 different mediums. Various media such as:

* Minimal medium broth
* Lactose broth
* Czapek–Dox medium
* Glucose salts broth
* MR-VP broth
* Mannitol salt broth
* Glycerol broth
1. OPTIMIZATION

After observation media will be optimized using one factor at a time approach.

1. BATCH FERMENTATION

After the media is optimized batch fermentation on lab scale will be done.

## DOWNSTREAMING

### RECOVERY

Filtration or Centrifugation of the substrate (Recovery of cell free filtrate)

1. PARTIAL PURIFICATION

Ammonium sulphate precipitation

* Protease can be precipitated from aqueous solution by addition of a cold acetone, ethanol, and isopropanol or ammonium sulphate.
* The ammonium sulfate concentration is increased stepwise, and the precipitated protein is recovered at each stage.
* Proteins are precipitated between 20%, 30%, 40%, 50% and 60% with ammonium sulphate saturation. Then protein fractions were dissolved in 2.5 ml 50 mM Tris-HCl buffer (pH-8) and dialyzed against same buffer. (For lipases 10–100%)

### CHROMATOGRAPHY FOR FURTHER PURIFICATION

Gel filtration (GF) chromatography separates proteins solely on the basis of molecular size. After getting our purified protease Sephadex G-100 gel filtration will be done.

# **COST\SCHEDULE**

As our project is still in its initial stages we cannot provide an accurate cost schedule but the estimated budget for the initial phase of the lab work will be around 8 to 10 lac.

# **CONCLUSION**

The production of microbial enzymes represents a promising path towards sustainable industries, addressing the environmental challenges posed by chemical-based practices. By replacing chemicals with microbial enzymes, industries can achieve several key benefits. Microbial enzymes are eco-friendly, biodegradable, and reduce pollution levels, improving air, water, and soil quality. They operate under milder conditions, enhancing energy efficiency and reducing greenhouse gas emissions. Their selectivity and specificity minimize waste generation and optimize resource utilization. The use of protease enzymes, a versatile microbial enzyme, further extends their applicability across multiple industries. Protease enzymes find applications in textiles, food and beverages, detergents, pharmaceuticals, and environmental sectors. They aid in processes such as bio finishing in textiles, flavor enhancement in food, stain removal in detergents, protein synthesis in pharmaceuticals, and organic matter degradation in wastewater treatment. Embracing the shift from chemicals to microbial enzymes offers sustainable alternatives, promoting cleaner production practices, reducing environmental impact, and contributing to a greener and more sustainable future.