Utilization of Agriculture Waste the Production of Food-Grade Enzymes

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ABSTRACT: ***In the age of global industrialization, enzyme are widely used in a variety of sectors, including food processing. Because enzymes are expensive, a novel approach for manufacturing low-cost enzymes was utilised. The study focuses on the utilization of Argo industrial waste in the production of enzymes. Using Aspergillus Niger and Aspergillus oryzae, an extracellular tannase was isolated from fermented agricultural waste such as red gramme husk, sugarcane bagasse, and rice straw. The synthesis of tannase was tested using solid-state fermentation (SSF) at varied pH levels or incubation periods. pH as well as incubation time were determined to be optimum at 5.5 and 72 hours, respectively. Purification of the crude enzyme using acetone extraction as well as DEAE cellulose chromatography yielded a purity of 21.36 times and a yield of 23 percent. The band of tannase at 72 kDa was identified by electrophoresis on an SDS–polyacrylamide gel. The natural form of the isolated enzyme was identified at 180 kDa during the gel localization investigation. The existence and location of the insoluble tannic acid – quinine combination were discovered, which was encouraging. An attempt was made to consume agricultural waste in order to produce enzymes for use in the food business[1]. This paper examines several types of agricultural industrial waste and how they may be utilised to manufacture enzymes. The present condition of enzyme utilization in the food industry has been investigated, as well as its future potential.***

KEYWORDS: ***Agriculture, Enzyme, Food, Product, Waste.***

1. INTRODUCTION

Concerns about pollution from agricultural and industrial wastes have motivated initiatives to convert waste materials into marketable goods. As a result of food production, preparation, and consumption, the Argo-food industry creates a considerable quantity of solid and liquid waste. In many cases, food processing wastes, in addition to being polluting and hazardous, may be repurposed as raw materials or transformed into higher-value products. Food-grade enzymes are widely employed in food processing (include preservation) and the manufacture of specialized components for formulation. Enzymes are biological catalysts that catalyze a wide range of biochemical processes, making them indispensable in many areas of life and activity[2]. Tannin acyl hydrolase (also known as tannase) is an enzyme that breaks down the ester link in hydrolysable tannins. Glucose and gallic acid are produced by a variety of filamentous fungi[3].

Several agro-industrial wastes and by-products can be utilised as enzyme substrates, include orange bagasse, sugarcane bagasse, wheat bran, red gramme husk, rice straw, and other food metabolites. It might also be used to clear instant wine, tea, drinks, fruit, and beer juices. In India, where the economy is mostly dependent on agriculture and agricultural techniques are intense the accumulation of agricultural waste is a major problem[4]. Every year, agricultural-based companies create a large number of residues. If these leftovers are discharged into the environment without being properly disposed of, they may pollute the ecosystem and threaten human and animal health. Because the majority of agro-industrial wastes are untreated and underused, they are often disposed of by burning, dumping, or unplanned landfilling. These untreated wastes contribute to climate change by increasing the amount of greenhouse gases released.

In these conditions, it was considered that searching for sources with tannase activity appropriate for commercial usage would be ideal. As a result, the current research focuses on the synthesis, partial purification, and characterization of tannase using Aspergillus Niger's and Aspergillus oryzae[5]. The first attempt to extract tannase from agricultural waste was performed based on the possible utility of tannase as a food-grade enzyme. We anticipate that our approach will be a cost-effective, stable alternative method that can be commercially exploited[6].

Since ancient times, microorganisms have been utilised in food fermentation, and fermentation techniques are currently used in the manufacture of numerous foods. Because microbial enzymes are more stable than plant and animal enzymes, they serve an important role in the food industry. They can be manufactured in a cost-effective way with minimal time and space requirements using fermentation processes, and because to their high consistency, process adjustment and optimization can be done relatively simply[7].

2. LITERATURE REVIEW

There are various researcher and researches who studies and analyses on effective utilizations of agricultural wastes for productions of food grade enzyme some of them are given below. Kanjan Upadhyay et al. studies the compounds generated on the ground as the seasons change are known as agricultural compounds. These chemicals are generated naturally and are necessary for the survival of animals and people who consume them. These plentiful chemicals can be utilised as a source of energy or converted into valuable commodities. In the related energy industry, crop waste has a significant potential for conversion to energy. From production through disposal, biomass is a waste product generated from animal waste or agricultural leftovers that has a physicochemical property and is interdependent with the environment. Previous research on biomass and agricultural waste conversion is examined in their study[8].

Parmjit S. Panesar et al. According to research, enzymes were widely employed in several fields, including food processing, throughout the time of global industrialization. The R&D industry has undertaken a number of projects to develop new techniques or enhance existing procedures for the manufacture of cost-effective enzymes, owing to the high cost of enzymes. As biotechnology advances, a variety of bioprocesses are being employed to manufacture different enzymes from various agro-industrial wastes. Their study focuses on agricultural and industrial wastes and how they may be used to make enzymes. They conclude that the growth of various technologies for the exploitation of waste for the manufacturing of value-added commodities such as enzymes has been fueled by regulations from various governmental and environmental bodies in response to the need for a cleaner environment. Of all the many forms of fermentation, batch fermentation has done the most work under solid state circumstances[9].

Pardeep Kumar Sadh et al. according to studies, agricultural wastes are abundant in bioactive compounds. These wastes may be utilised as a raw material in a number of studies and businesses to create products such as biofuel, biogas, tempeh, and mushrooms. The use of agro-industrial waste as a raw material has the potential to lower production costs while also lowering pollution levels. From agro-industrial waste, solid state fermentation is utilised to create enzymes, biofuels, antioxidants, vitamins, animal antibiotics, feed, and other chemicals (SSF). A number of microorganisms are used in SSF techniques to produce these valuable chemicals. As a consequence, the SSF and its influence on the creation of a value-added product are investigated and assessed[10].

Research Questions:

- What is the most efficient way to use agricultural waste?
- What factors should be considered while using agricultural waste?

3. METHODOLOGY

3.1. *Design:*

Sun dried sugarcane bagasse, rice straw, and red gramme husk were crushed and kept in airtight containers. Potato dextrose agar slants sporulation medium was used to produce spores of A. Niger's and A. oryzae. Inoculated with 1 mL Aspergillus Niger spore inoculums, the solid substrates were incubated for 70 hours at 37°C. Tannase was extracted by adding citrate buffer to the incubated substrate as well as centrifuged at 7000 rpm for 15 minutes at 5°C before being filtered. The filtrate was collected and stored at 5°C.

3.2. Instrument:

Lowry's technique was used to calculate protein. Tannase activity was measured at 310nm using a spectrophotometric technique. At a rate of 1ml/10mins, dialyzed diluted sample was put to DEAE cellulose chromatography column. The approach of gel localisation of tannase was followed. 1 mL of tannase (100U/mL) was added to 05 mL of juice.

3.3. *Data Collection:*

Sigma Compounds, Merk, and SRL provided the chemicals used in the research. Real Foods sells canned apple juice (Dabur food products). Local markets in Kolkata provided fresh apple, red gramme husk, rice straw, and sugarcane bagasse. Aspergillus Niger from the college lab as well as Aspergillus oryzae (MTCC 634) from IMTECH in Chandigarh.

3.4. *Data Analysis:*

After 72 hours at 37°C and pH 5.5, optimum tannase production was reached. Reduced medium levels can affect enzyme activity or synthesis, resulting in a reduction in enzyme activity. In comparison to Aspergillus oryzae and sugarcane bagasse, tannase produced from the fermenting mass including red gramme husk and Aspergillus Niger was shown to be higher, rice husk. The activity of the crude extract was measured at 14.4 U/ml. The activity of extracellularly generated crude tannase is similar to that previously reported. To test the viability of red gramme husk as a fermented substrate, it was infected with Aspergillus oryzae at pH 5.5 for 72 hours. The activity of this fermenting mass was 12U/ml (Figure 1). The increased activity is due to the red grame husk's dual roles as a source of nutrients for the microbial culture and a source of maximal enzyme synthesis.

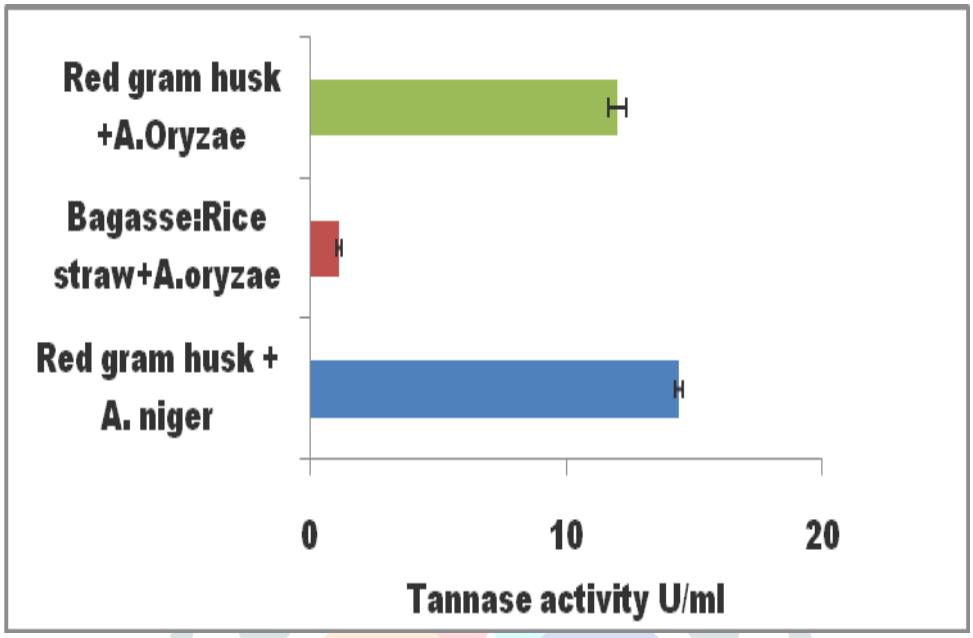

Figure 1: Various Fungal Strains Produce Extracellular Tannase Aspergillus Oryzae Fermented with Sugarcane Bagasse: Rice Straw, and Aspergillus Niger Fermented with Red Gram Husk T=370°C; PH=5.5; 72 hours The standard deviations of the triplicate findings are represented by the error bar.

4. RESULTS AND DISCUSSION

Tannase was purified using DEAE-cellulose column chromatography (Figure 2), resulting in a purification yield of 21.3 folds and a yield of 23%. The 23 percent tannase yield produced in this study was greater than the 20 percent yield reported by others.

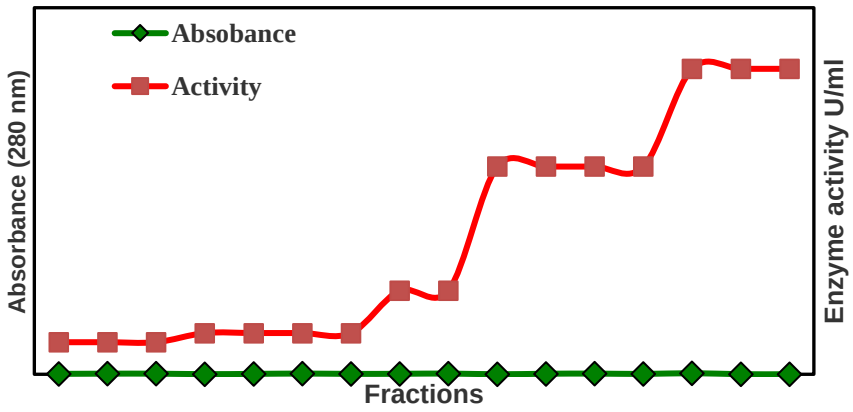

Figure 2: Tannase Produced by A. Niger Elution Profile on DEAE Cellulose Using Nacl Solution as Eluant The Enzyme Solution was obtained via Acetone Precipitation and concentrated using Centric on Membrane Ultra fiction. It was then loaded onto a DEAE Cellulose Column equilibrated in 0.2-M Acetate Buffer, PH 5.0. The flow rate was increased to 1 ml/min, and 0.5-ml fractions were eluted with a linearly increased molarity Nacl solution (0.1 To 1).

Tannase comprising fraction were pool and were subjected to SDS PAGE. Purified enzymes migrates as unique protein bands corresponding to molecular masses of 72 kda (Figure 3).

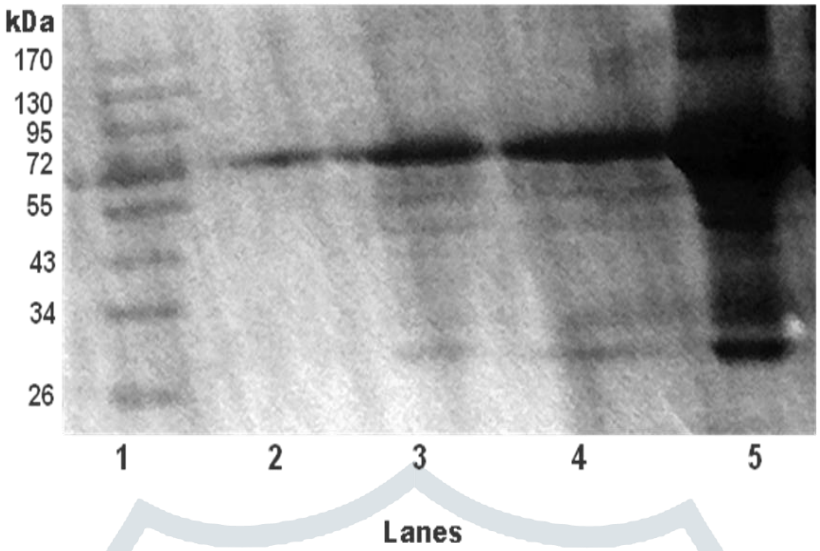

Figure 3: SDS –PAGE Showing The Molecular Weigh Marker , Crude Extracts, Acetone Precipitated Sample , Centricon Concentration Sample (Lane 4), After Purification By DEAE –C Column (Lane 5). *The SDS-PAGE* Analysis Shows Different Fraction Of Enzyme Obtained Through Subsequent Purification.

The native tannase detection technique revealed that the native pure tannase is a 180 kDa dimeric protein (approximately). The insoluble tannic acid – quinine combination provided the gel a white coloration with a clear band, indicating that tannase was present and in the right place (Figure 4).

Figure 4: Tannase activity is shown on a gel. DEAE-C sample, crude extracts, acetone extraction fractions, and protein marker (lane 4). The presence of native tannase with a molecular weight of 180 kDa (approx) is shown by the fourth lane.

After 2 hours of treatment with pure tannase at 37°C, the tannin level of fresh apple juice was reduced by 81 percent (Figure 5). Purified tannase's impact in packed apple juice was also studied (Figure 6). This research backs up earlier studies that shown that pomegranate and anole juice reduced tannin levels by 35 and 24 percent, respectively. The current study resulted in a greater proportion of tannin breakdown (81%) and hence aimed to be more efficient for bitterness reduction.

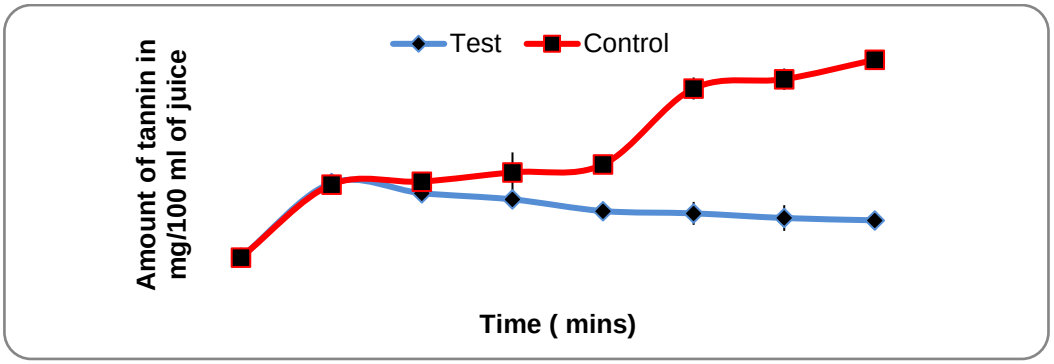

Figure 5: Effect of Tannase (1ml and 100 U/ml) on tannin breakdown in fresh apple juice at various periods. The standard deviations of the triplicate findings are represented by the error bar.

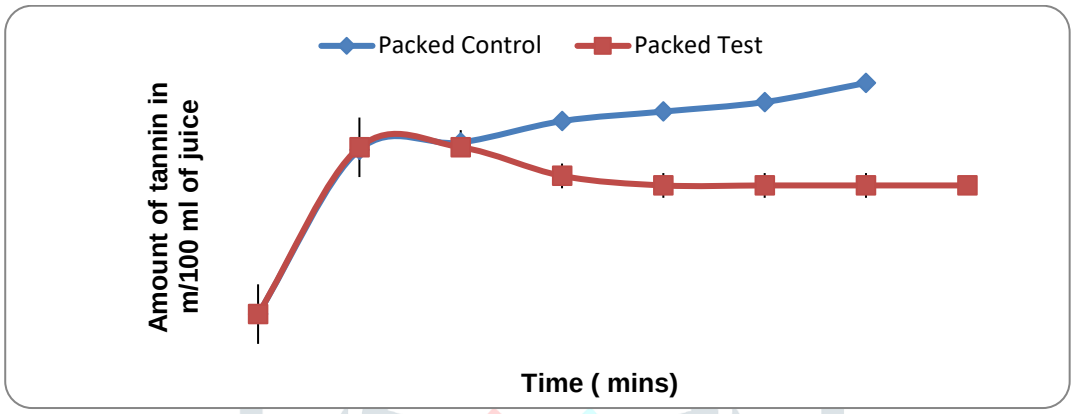

Figure 6: Effect of Tannase (1ml and 100 U/ml) on tannin breakdown in Packed apple juice at various periods. The standard deviations of the triplicate findings are represented by the error bar.

The optimal tannase production was achieved after 72 hours at 37°C and pH 5.5, according to this article. Reduced medium levels may have an impact on enzyme activity including synthesis, resulting in a decrease in enzyme activity. Rice straw, tannase produced from the fermenting mass including red grame husk, and Aspergillus Niger were found to be larger than Aspergillus oryzae and sugarcane bagasse. Tannase was purified using DEAE-cellulose column chromatography, resulting in a purity of 21.3 times and a yield of 23%. This study's 23 percent tannase yield was greater than the 20 percent yield reported by others.

The tannase-containing fractions were combined and subjected to SDS PAGE. From the isolated enzyme, a single protein band with a molecular mass of 72 kDa migrated. The natural pure tannase is a 180 kDa dimeric protein, according to the native tannase detection technique (approx.). The insoluble tannic acid – quinine combination gave the gel a white hue and a distinct band, showing that tannase was present and in the correct location. The tannin content of fresh apple juice was decreased by 81 percent after two hours of treatment with pure tannase at 37°C. The impact of purified tannase in packed apple juice was also investigated.

Concerns over contamination from agricultural and industrial wastes have prompted initiatives to turn waste materials into marketable commodities, according to this article. The Agro -food sector creates a significant quantity of solid or liquid waste as a result of food production, preparation, and consumption. Food processing wastes may often be reused as raw materials or converted into higher-value products, in addition to being polluting and dangerous. Enzymes are biological catalysts that catalyze a wide range of biochemical reactions, making them essential to a variety of human activities. As a result, this article is utilised to effectively utilize agricultural wastes for the manufacturing of food-grade enzyme.

5. CONCLUSION

Concerns about contamination from agricultural and industrial wastes have prompted efforts to turn waste into commercial commodities. The Argo-food business generates a significant amount of solid and liquid waste as a result of food production, preparation, and consumption. Food processing waste may often be reused as raw material or converted into higher-value products, in addition to being polluting and dangerous. Food-grade enzymes are commonly used in food processing (including preservation) and the production of specific formulation components. Enzymes are catalysts that carry out a variety of biochemical reactions and hence play a vital part in many aspects of life and activities. They are biodegradable and environmentally

benign, act by increasing the pace of any reaction by lowering the activation energy, and contribute to lower manufacturing costs by reducing resource needs. This study emphasizes the potential of agricultural waste as a source for microbial production of food-grade enzyme. Enzymes have long been a vital part of human existence for the production of a variety of food items, and as a result, they are extensively investigated in the food industry. Using agricultural wastes for microbial fermentation can improve procedure economics while also lowering environmental concerns about waste disposal. This paper examines several types of agricultural industrial waste and how they may be utilised to manufacture enzymes. The present condition of enzyme utilization in the food industry has been investigated, as well as its future potential.

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