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Agro-industrial residues as solid substrate for α-amylase production by bacillus licheniformis

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Abstract

Solid dry wastes are used as fermentation substrates in numerous industries these days, including textile, culinary, and medicinal. Solid surface matrix is employed in solid-state fermentation (SSF), a fermentation process that occurs without or almost entirely with the availability of free water. This involves growing microorganisms without an aqueous phase that is free to flow. SSF has now taken the place of submerged fermentation (SmF), which was a conventional method for producing α-amylase. SSF is used as an alternative to SmF in many industrial processes, including the synthesis of enzymes, biofuels, organic acids, single-cell proteins, antibiotics, fragrances, and biopesticides. In the past, fungi were the only microorganisms of option for SSF fermentation operations; however, these days, a range of bacterial strains are also employed in them. Because of its simplicity, comparatively low initial and ongoing costs, reduced water production, and other advantages, SSF improves the natural habitat of microorganisms and is a better option overall. SSF containing agro-industrial leftovers has also taken the place of expensive media used in submerged fermentation to produce α-amylase. An other source for using these substrates as a solid substrate for the synthesis of amylase and other beneficial industrial products is the filamentous fungus that consumes agro-industrial residues. In this research, we attempted to examine the potential and optimization techniques of several solid substrates employed in the synthesis of fungal amylase.

Keywords: Wastes, fungus, agro-industrial residues, enzymes, biofuels, organic acids

1. Introduction

α-amylases are widely utilized enzymes among the many vital enzymes for industry; they are necessary for the fermentation of various foods. In addition to the food and starch industries, α-amylases find extensive use in paper pulp, textile, and other related industries. Amylase enzymes are used in a wide range of industrial processes, such as the synthesis of cyclodextrins and the conversion of starch to sugar, corn, and maltose syrup for food. The demand for amylases in the paper and pulp, textile, and other industries is rising. Of all the enzymes produced worldwide, these enzymes make up around 30%. There is a great deal of interest in creating amylase with improved qualities, thermal stability, and suitability for industrial use by adopting cost-effective production procedures due to the growing demand in a variety of industries (Pandey *et al*., 2003) [12]. Microorganisms, plants, and animals manufacture α-amylase, which is essential for the metabolism of carbohydrates. Since ancient times, food additives derived from plants and microorganisms have been utilized. Fungal amylases are extensively utilized in the processing and preparation of oriental foods, whereas amylases derived from barley have found application in the brewing industry. According to fungal amylases are frequently used in the processing and preparation of oriental meals, while barley amylases are employed in the brewing industry. Because amylases from microbial sources are consistent, easy to modify and optimize processes for, and stable at high temperatures, they are used in the industrial production of many important goods. Amylases are produced by bacteria, plants, and animals alike and are essential to the metabolism of carbohydrates. Since ancient times, food additives derived from plants and fungi have been utilized. α-amylase is produced by filamentous fungus like Aspergillus and bacteria. Nonetheless, it has been discovered that the fungal amylase is more stable than the bacterial enzyme. Fungal filamentous matter is a desirable substrate for the synthesis of economically valuable enzymes. Using bread trash as a solid substrate, Rhizopus oryzae coproduces the industrially significant enzymes amylase and protease. The effects of several physical and chemical factors were investigated.
Bacillus subtilis, a bacterial strain, is used in the synthesis of

amylase utilizing solid residues like wheat bran, rice bran, banana peels, and other fruit peels to maximize enzyme production while requiring the least amount of additional carbon and nitrogen sources. Using the edible fungus Neurospora intermedia, the combined effects of submerged and solid state fermentation were investigated for the biotransformation of ethanol. This research may be helpful for the manufacture of additional industrially significant products.

Agro-industrial solid wastes from all of these fermentation processes can be effectively utilized as solid substrates for SSF. This review paper aims to assist the scientific community by providing an overview of the data on SSF for α-amylase synthesis that is dispersed among different literatures. For this aim, a variety of solid substrate types are employed, each with unique advantages and disadvantages. As a result, this article will provide researchers with information about the potential of the many solid substrates employed for fungal amylase production as well as optimization techniques.

1.1 Role of filamentous fungi for amylase production

Researchers are always looking for new fungal strains to isolate for the purpose of producing amylase, even though many fungal strains are currently employed commercially. Researchers from Brazil have discovered a novel fungus in the Atlantis-forest region that can produce more glucoamylase when given uniform circumstances and a solid substrate made of agro-industrial waste. When 1% starch was added to the media, the isolated fungus A. carbonarius produced the most amylase at pH 6.0, 30 ºC, and 96 hours of incubation.

Due to their highly effective enzymatic systems, fungi are able to break down lignocellulosic waste and produce a wide range of valuable compounds that could be used in the manufacturing of numerous useful goods. Fungi are known to possess two distinct types of extracellular enzymatic systems: hydrolytic and ligninolytic systems. According to Abdel-Azeem *et al*. (2020) [1], lignocellulosic residues from urban and agricultural solid wastes are very common in nature and have the ability to undergo bioconversion.

For the past few decades, SSF has been used to produce α-amylases and other industrially significant enzymes, amino acids, antibiotics, and organic acids, thanks to filamentous fungi's exceptional and unique ability to digest cellulosic waste. As these moulds are recognized for the synthesis of extracellular compounds such as enzymes. Thus, filamentous fungi are extensively utilized in a number of SSF processes to produce α-amylase and other industrial products. Solid-state fermentation (SSF) employing agricultural wastes and molds to produce enzymes and other industrially significant products has shown to be an economical method of production; unexpectedly, most of these processes are optimized. Comprehensive literature is accessible on the several fungal sources that produce amylases.

1.2 Agricultural by products in amylase production

Compared to plant and animal enzymes, microbial enzymes are more adaptable, stable, and have a broad range of industrial uses. Given the high concentration of cellulosic material and other physiologically active substances present in these agricultural leftovers. Consequently, these agricultural wastes can be utilized as a substitute solid substrate in SSF for the synthesis of industrially significant compounds, such as animal feed, biofuel, and mushroom cultivation, in addition to industrial products like enzymes, organic acids, and amino acids, among others.

These agro-industrial residues, which include molasses, rice husks, rice bran and wheat bran, bagasse, leaves, straw, stalk, shell, pulp, peel, roots, and so on, can be utilized in industrial processes to produce high-value industrial products while lowering production costs and the environmental pollution load. These substrates have long been utilized for biofertilizers, animal feed, and improving soil quality. However, a significant amount of field residue is produced as a result of scientific advancements and technological advancements in agricultural techniques. The improper and non-eco friendly practice of disposing of unused agricultural waste is troublesome. Massive amounts of processed leftovers, or oil cake, are created in the oil exploration sectors. High concentrations of fat, suspended carbohydrates, and dissolved solids are all found in these residues. There are several varieties of oil cakes, including those made using canola, sunflower, coconut, sesame, mustard, soy, and groundnut oils (Ramachandran *et al*., 2003) [12]. Various agricultural byproducts have been utilized as solid substrates to produce a range of industrial goods, such as fermentation process enzymes.

2. Materials and Methods

2.1 Bacterium selection & growth conditions

The Bacillus licheniformis, strain MTTC 1483, was obtained from the Institute of Microbial Technology, and located in Chandigarh, India. In soup infused with brain and heart, the strain was revitalized. The bacteria were regularly sub-cultured every three days or as needed, and it was then kept in storage at 4 °C.

2.2 Amylase activity

The bacterial strain underwent a starch hydrolysis test to determine its capacity to make α-amylase. By adding Gram's iodine solution to the plate, one could see the zone of hydrolysis that developed on the starch agar plates. To confirm the generation of amylase, the zone of inhibition was examined.

2.3 Substrate collection

Four different types of agricultural wastes namely sugarcane bagasse, wheat straw, paddy straw and maize straw were selected as substrate for producing α- amylase by SSF.

2.4 Enzyme assay

Using the Dinitrosalicylic technique, amylase was measured in the supernatant containing crude enzyme, and an ultraviolet spectrophotometer was used to measure optical density at 540 nm. This method used 1% starch produced in 50 mM phosphate buffer as the substrate and 1 ml of enzyme extraction as the enzyme. The prepared mixture was incubated for 10 minutes at 50 °C. A 2 ml DNS reagent was added following incubation. To produce color, the incubation was repeated for five minutes at boiling temperature. To complete the DNS reaction, 1 milliliter of 30% potassium sodium tartrate was then added. At 540 nm,

absorbance was measured. The amount of product produced by one milliliter of enzyme using one gram of solid substrate in one minute is known as one unit of enzyme activity.

3. Results and Discussion

Amylases (E.C. 3.2.1.1.) are one of the most widely utilized enzymes in industry and account for 25% of the global enzyme market. The growing need for amylase in the food, beverage, and textile industries has brought attention to the need for better enzyme production technology. Recent research has focused on the use of different agro-industrial wastes to generate higher quantities of enzymes at comparatively low costs. Using substrates like wheat straw, paddy straw, sugarcane bagasse, maize straw, etc., enzymes are generated using SSF, a cutting-edge method. Environmentalists are quite concerned about the disposal of agro-industrial waste since burning this trash produces a lot of pollution. Not only are these wastes rich in nutrients, but their poor digestion makes them unsuitable for use as animal feed. Reusing wastes as a substrate for the manufacture of enzymes is an alternative to burning and discarding them. The goal of the current study was to optimize the fermentation parameters for increased amylase production through solid substrate fermentation in light of the benefits of amylase.

3.1 Analyzing the amylase producing capability of b. *licheniformis* MTCC 1483:

IMTECH Chandigarh provided B. licheniformis MTCC 1483, which was then brought back to life in a soup infused with brain and heart. Gram-positive, non-capsulated rods were visible in light microscopic pictures of the cells. The strain's morphological features. The obtained strain sporulated rods measuring 0.6–1.0 µm in size and was Gram positive. The starch hydrolysis test was used to evaluate B. licheniformis MTCC 1483's capacity to produce amylase. The primary substrate that amylase uses is starch. With two components, amylose and amylopectin, it is a complex carbohydrate. α-D-glucose molecules are linked by α-1, 4-glycosidic links. Due to its size, starch is unable to pass through the membrane of bacterial cells. Starch is hydrolyzed by amylase into glucose and maltose. Through a semi-permeable membrane, these simplest sugars can reach the cytoplasm of the bacterial cell and be used for cell growth.

The genus Bacillus is known to produce a wide variety of enzymes, the most important of which is amylase for industrial use. A microbe's capacity to break down starch is typically used as a criterion to assess if it is producing amylase. In this test, iodine is employed as an indication. The starch traps iodine, giving it a dark blue tint. A distinct halo surrounding the colony indicates the area of amylolytic activity.

Table 1: Morphological Characteristics of *Bacillus licheniformis*

Test	Result
Gram staining reaction	Gram +ve
Appearance	Rod shaped
Spore	Central to para-central, ellipsoidal to cylindrical
Arrangement	Placed singly (sometimes chains and bunches)
Size	0.6-1.0 µm in length
Capsule formation	No capsule

Table 2: Production of amylase by SSF using different substrates

Sr. No.	Substrate	Amylase production (IU/g)
1	Sugarcane baggase	0.620±0.020
2	Wheat straw	0.600±0.010
3	Paddy straw	1.004±0.020
4	Maize straw	0.770±0.010

3.3 Effect of Incubation Time on Amylase Production

It was discovered that as the culture grows, the production of α-amylase increases. At 24 hours, the activity peaked, and then it started to diminish. The findings indicate that the bacteria release α-amylase during the logarithmic or growth phase of the culture to make use of the carbohydrates in the paddy straw. According to published reports, bacteria create amylase as they are growing. Numerous writers have said that bacteria can produce an item in two to three days. In the current study, shorter fermentation times are beneficial since they result in lower power usage and production costs.

Table 3: Effect of incubation time on amylase production

Sr. No.	Incubation Time in hrs.	Enzyme Production (IU/g)
1	12	0.06000±0.01
2	24	1.30000±0.02
3	48	0.34000±0.02
4	72	0.09000±0.01

4. Conclusion

The abundance of nutrients found in agro-industrial waste, along with the presence of bioactive chemicals, promote the growth of filamentous fungus during solid state fermentation. These agro-industrial residues are used as raw materials in bio-conversion processes to produce high-value industrial goods since their composition includes sugars, minerals, proteins, and amino acids, among other things. Due to their special abilities, the microbes can grow and feed on these solid substrates during fermentation operations, which produces a wide range of industrially useful biochemical products. SSF is a more straightforward, yet economical, method for bioprocessing and industrial scale optimization. As an alternative, using agro-industrial wastes as raw materials can lower manufacturing costs and improve environmental sustainability through waste recycling. Of all the enzymes, amylase is the one that is most frequently employed for both medical and industrial applications. The production of amylase on a large scale for cost reduction and effective use of agro-waste is being led by the development of new and innovative techniques in bioprocess engineering, such as Response Surface Methodology (RSM), Artificial Neural Network (ANN) based optimization, and Bioreactor designing for SSF. Establishing a large-scale fermentation process with the right fungus strain will be made easier by this review.

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