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A Comparative Study Between Soluble and Immobilized Enzyme by Using Solid State Fermentation Technique

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Abstract

Microbial amylases are enzymes produced by microorganisms to hydrolyse starch. There are three types of microbial amylases, namely: alpha-amylase, beta-amylase, and glucoamylase. Each of these amylases has a unique way of acting on starch to yield simple glucose monomers. Microorganisms, plants, and animals are sources of amylases but much attention is given to microorganisms since the amylases produced by them have greater thermal stability as well as give rise to different sugar profile thus meeting industrial demands. In the present study amylase production was optimized using Solid Substrate fermentation after screening of six types of agro industrial waste material as substrate i.e. . Banana peel, Orange peel, Pineapple peel, Papaya peel, Potato peel and Sugarcane Bagasse. The results obtained in the present study indicated that culture DM01 is a potential strain for amylase production using solid-state fermentation with pineapple peel as substrate. Various production parameters like temperature, Ph, thermostability and incubation period were also optimized. Immobilization and reusability of enzyme were analysed. The results obtained shows that enzyme isolated from pineapple peel is more active in soluble as compared to its immobilized forms also the activity gradually decreases till the three washes.

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Introduction

Amylases is a group of enzymes that hydrolyzeglucosidic bonds present in starch, namely, α-amylase, β-amylase, and glucoamylase.Amylase is present in the saliva of humans and some other mammals, where it begins the chemical process of digestion. The pancreas and salivary gland make amylase (alpha amylase) to hydrolyse dietary starch into disaccharides and trisaccharides which are converted by other enzymes to glucose to supply the body with energy.

Plants and some bacteria also produce amylase. As *diastase*, amylase was the first enzyme to be discovered and isolated (by AnselmePayen in 1833).

Microbial amylases are enzymes produced by microorganisms to hydrolyze starch. There are three types of microbial amylases, namely: alpha-amylase, beta-amylase, and glucoamylase. Each of these amylases has a unique way of acting on starch to yield simple glucose monomers. Microorganisms, plants, and animals are sources of amylases but much attention is given to microorganisms since the amylases produced by them have greater thermal stability as well as give rise to different sugar profile thus meeting industrial demands. Two major groups of microorganisms play pivotal role in amylase production, namely: bacteria and fungi.Amylases are obtained from various origins like plant, animal, bacterial and fungal. Several researchers produces amylase enzyme using Bacillus sp. .There are about 3000 enzymes known today. These are mainly extracellular hydrolytic enzymes, which degrade naturally occurring polymers such as starch, proteins, pectin s and cellulose .In the production of glucose syrup the amylase is used in the first step of enzymatic degradation yielding a mixture of glucose and fructose with high fructose content. The amylases can be derived from several sources such as plants, animals and microbes. The microbial amylases meet industrial demands because it is economical when produced in large quantities . Amylase has been derived from

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several fungi, yeasts, bacteria and actinomycetes, however, enzymes from fungal and bacterial sources have dominated applications in industrial sectors.

An extra-cellular amylase, specifically raw starch digesting amylase has found important application in bioconversion of starches and starch-based substrates. The level of alpha amylase activity in various human body fluids is of clinical importance e.g. in diabetes, pancreatitis and cancer research, while plant and microbial alpha amylases are used as industrial enzymes. Starch-degrading amylolytic enzymes are of great significance in biotechnological applications ranging from food, fermentation, textile to paper industries. Although amylases can be derived from several sources, such as plants, animals and microorganisms, the enzymes from microbial sources generally meet industrial demands and had made significant contribution to the production of foods and beverages in the last three decades. The microbial amylases have almost completely replaced chemical hydrolysis of starch in starch processing industry.

Materials And Methods

Collection of soil sample

Soil sample was collected from different regions of Indore.

Isolation of Organism

Starch agar is a selective medium was used for the isolation of Amylase producing organisms. About 1.0gmof soil sample was serially diluted in 10 ml of sterile distilled water. 0.1ml of sample from each dilution was spread onsterile Petri dishes containing starch agar with the help of wire loop and the plates were incubated at 37°C for 24-48 hours. After incubation the plates were observed for the growth of bacteria.

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Determination of amylase activity

Al1 the Bacterial isolates were tested for amylase production by starch agar. Starch agar medium were spot inoculated with the organism and subsequently flooded with iodine solution. Cleared zone are seen around amylase producing colonies under blue background. The zone diameter weas taken for each amylase producing isolate.

Characterization of Bacterial isolates

The colonies grown on starch agar plates were subjected morphological analysis by using Gram's Staining.

Agro-Waste as Substrates for Amylase Production

Banana Peel, Orange peel, Pineapple peel, Papaya Peel, Potato Peel and Sugarcane Bagasse were chosen as a substrate for the production of bacterial amylase by SSF. They were procured from local juice vendors of Indore. Substrates were dried and ground in the grinder to make small particles.

Fermentation Medium

Solid-state fermentation (SSF) was carried out in 250 ml Erlenmeyer flasks containing 5 g of each substrate i.e .Banana Peel, Orange peel, Pineapple peel, Papaya Peel, Potato Peel and Sugarcane Bagasse. The substrate was moistened with 10 ml of distilled water (KH2PO4 0.1g/L, NaCl 0.25g/L, MgSO4 0.01g/L, CaCl2 0.01g/L) autoclaved at 121°C for 15 min and cooled. The flasks were inoculated with 1% (v/w) bacterial inoculum and incubated at 37°C for 4days

Enzyme Assay

Amylase was assayed using supernatant containing crude enzyme by Dinitrosalicylic method (Miller, 1959) and optical density was taken at 540nm,

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on UV- spectrophotometer. For this method the enzyme was extracted by using Phosphate buffer as the buffer was added with the inoculum at the fourth day of incubation and incubated for 1 hrs in shaking condition. The production medium was then centrifuged at 10,000 rpm for 10 mins. 1 ml enzyme extraction was taken and 1% starch and 1 ml phosphate buffer. Mixture was incubated at 50 C for 30 min. After incubation added 2 ml DNS reagent and again incubated at boiling temperature for 5 min cooled at room temperature. After that add 1 ml potassium sodium tartrate and made up the final volume up to 10ml with distilled water and took the absorbance at 540nm.Blank was prepared by without adding starch.

Enzyme Immobilization

Entrapment of enzyme in calcium alginate beads was studied. The crude enzyme preparation was mixed with 2% sodium alginate prepared in 0.1M sodium phosphate buffer (pH 7.0). This mixture was dropped into 0.05M calcium chloride with stirring at room temperature. Allow the beads to for complete gelation. After this, enzyme assay has been performed.

Enzyme Reusability

The gelatine beads were washed with distilled water after the enzyme assay and allow it o dry. After the enzyme assay has been again performed. The procedure continues till the optical density decreases.

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Results

Isolation and Characterization of Organism

Out of 23 Isolates DM01 produces maximum zone of clearance and serves as the best isolate for this study. The selected strain was Gram Negative bacteria.

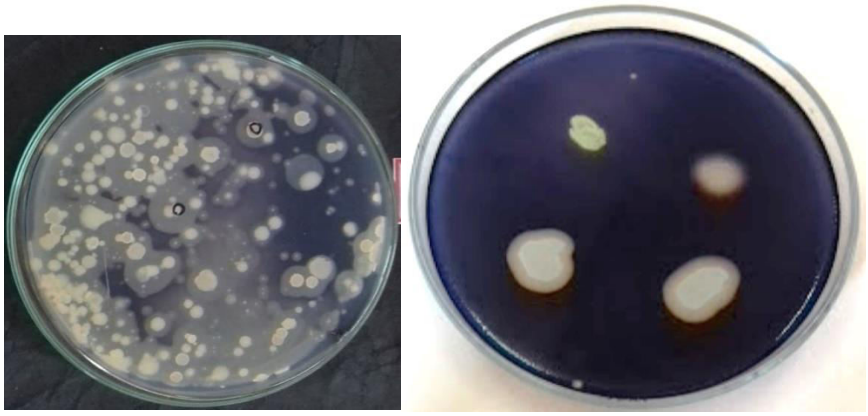

Fig- Primary screening of Amylase producing organism

Enzyme Assay

From the present study it was concluded that the maximum absorbance was observed in pineapple peel.

Table 1- Activity of DM01 on Different Substrates.

S.No.	SUBSTRATE	OPTICAL DENSITY
1	Papaya peel	1.02
2	Pineapple peel	1.34
3	Orange peel	0.56
4	Potato peel	0.58
5	Banana peel	0.72
6	Sugarcane Bagasse	0.43

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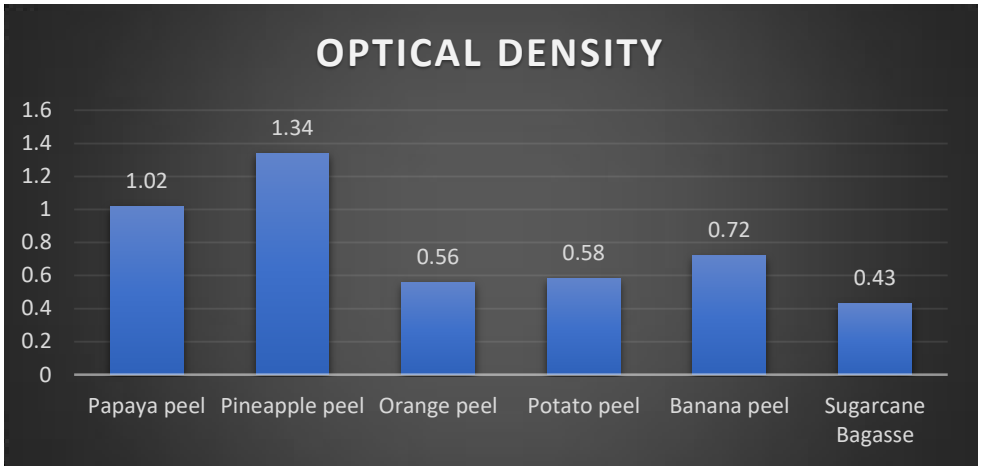

Fig 01- Activity of DM01 on Different Substrates.

Immobilized Enzyme Activity

From the present study it was concluded that Pineapple shows the maximum activity in immobilized forum as compared to other substrates.

Table 02- Enzyme Activity in Immobilized Foum

S.No.	SUBSTRATE	OPTICAL DENSITY
1	Papaya peel	0.96
2	Pineapple peel	1.12
3	Orange peel	0.48
4	Potato peel	0.52
5	Banana peel	0.60
6	Sugarcane Bagasse	0.38

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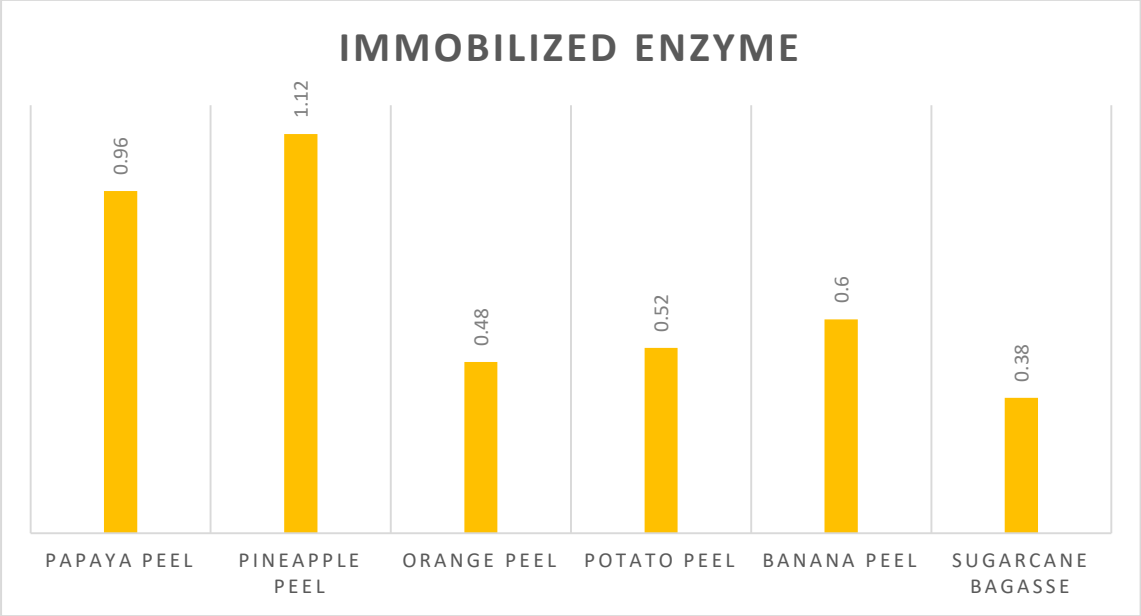

Fig 02- Activity of Enzyme in Immobilized fourm

Comparison Between Soluble And Immobilized Enzyme

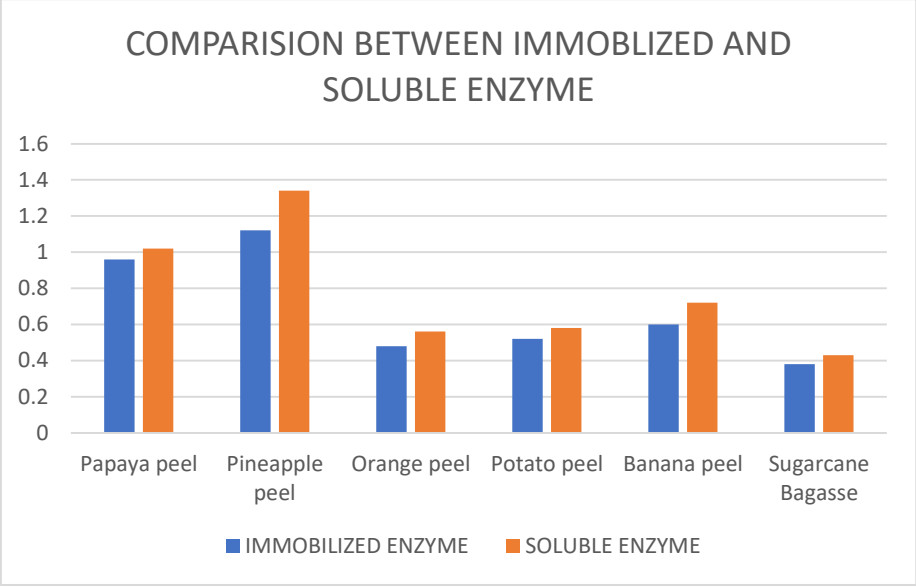

Fig 03- Comparision Between Immobilized And Soluble enzyme.

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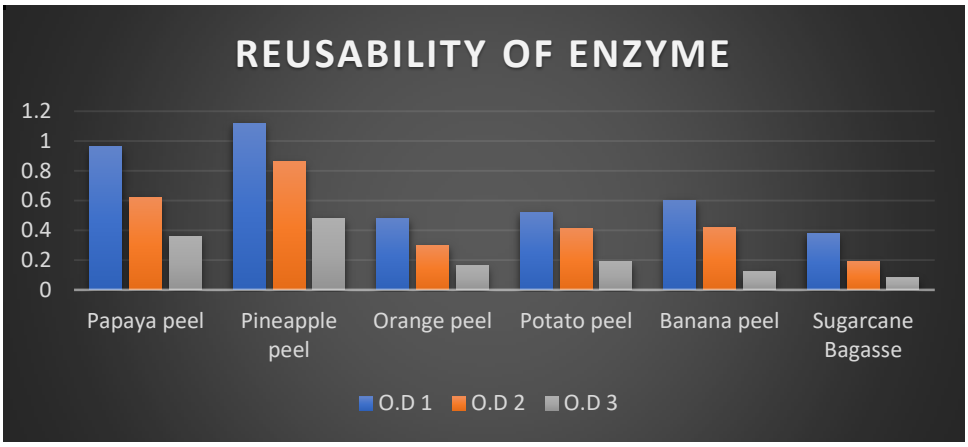
Reusability Of Enzyme

S.No.	SUBSTRATE	O.D 1	O.D 2	O.D 3
1	Papaya peel	0.96	0.62	0.36
2	Pineapple peel	1.12	0.86	0.48
3	Orange peel	0.48	0.30	0.16
4	Potato peel	0.52	0.41	0.19
5	Banana peel	0.60	0.42	0.12
6	Sugarcane Bagasse	0.38	0.19	0.08



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