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Production and characterization of industrially important amylase enzyme by *Aspergillus niger* using different combination of starch waste as substrate

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Abstract

The continued development of biosustainable and renewable resource technology is of great importance with respect to environmental concerns. The present work was carried out to comparatively see the production of amylase in medium where different combinations (1:1,2:2,3:1) of agro-wastes like banana peel, orange peel, sweet lime peel, papaya peel, potato peel, wheat bran and sugarcane bagasses were used in powder form in the production media instead of starch. Out of 12 combination of agrowaste papaya and orange (2g:2g) showed maximum production of amylase enzyme (46U/ml/min) ,orange and sweet lime (2g:2g) 45.33U/ml/min,papaya and potato peel (2g:2g) 38.33U/ml/min and wheat bran and sugarcane bagasses (1g:3g) 24U/ml/min showed least enzyme production as compare to other agrowaste combination. The highest enzyme activity was observed at pH 7.0, optimum temperature 70℃ and optimum substrate concentration was found to be 3% of starch.

Keywords: Amylase, Agrowaste, DNS.

1. INTRODUCTION

Agro-industrial wastes are generated during the industrial processing of agricultural or animal products the chemical composition of these kinds of wastes shows that these are usually rich in sugars, minerals and proteins, and hence, these can be used as raw materials for other industrial processes. The major advantage of these is that they are abundant and renewable. The carbon sources, nutrients and moisture present in such wastes provides suitable conditions for the growth of microorganisms, and hence these wastes can be used as solid support, carbon or nutrient source for the production of a variety of compounds which are of great value [1-3]. The main products which can be produced are enzymes, ethanol, furfural, reducing sugars, protein and amino acids, secondary metabolites, carbohydrates, lipids, organic acids, surfactants phenols, activated carbon, methane, degradable plastic composites, cosmetics, resins, medicines, foods and feeds, biosorbent, biopesticides, biopromoters, fertilizer and other miscellaneous products [4-8].

2. MATERIALS AND METHODS

2.1. Collection of agrowaste samples

Samples of agro-waste products i.e., peel of potato, orange, papaya, sweet lime, banana, wheat bran and sugarcane baggases were collected from juice shops and domestic sources. These peels were left to dry under sunlight for 10-15 days. Once completely dry and devoid of moisture, potato peels, orange peels, sweet lime peels and papaya peels were turned into powder form using a grinder and further sieved to get finer powder. The powder form of every sample was stored in air tight containers for further use. Sugarcane baggases was first treated with 1.0% NaOH to get rid of lignin content present in sugarcane. Sugarcane baggases was soaked in 1.0% solution of NaOH solution for overnight. Then it was washed with distilled water 2-3 times and left to dry in the sun.

2.2. Screening of amylase producing fungal strain

Fungal colonies were isolated from soil samples by serial dilution method. 50µl of soil samples diluted up to 10^{-5} dilutions were plated on starch agar medium. After seven days of incubation at 28°C the Grams iodine solution (for amylase) is added to the cultures plates, plates showing highest zone of clearance were selected for amylase production.

2.3 Preparation of production media

Production Media was comprised of (g/l); Peptone 5g, NaCl 5g, Beef extract 1.5g, Yeast

extract 1.5g and different combination of agrowaste in 1 L of distilled water instead of starch. Different agrowaste ratio in grams used in production media are as follows; Orange: Sweet lime(1;3.2;2,3;1), Orange:Papaya(1;3,2;2,3;1), Sweetlime: Banana(1;3,2;2,3;1), Orange:Banana(1;3,2;2,3;1), Papaya:Sweet lime(1;3,2;2,3;1), Orange:Potatopeel(1;3,2;2,3;1), Sweetlime:Potato peel(1;3,2;2,3;1), Papaya: Potato peel(1;3,2;2,3;1), Wheat Bran: Orange(1;3,2;2,3;1), Wheat Bran: Sweet lime(1;3,2;2,3;1), Wheat Bran: Papaya (1;3,2;2,3;1), Wheat Bran: Sugarcane baggases (1;3,2;2,3;1). Once 100 ml of media with each of the above agro-waste combinations was prepared, the medium were autoclaved.

2.4. Crude enzyme extraction

Each flask was inoculated with screened culture of *Aspergillus niger* in laminar air flow using inoculation loop. The flasks were incubated at 30℃ for 3 days. After 3 days, a clear mat of A. niger was seen on top of the production media in the flask. It was then filtered through Whattman filter paper.

2.5 Enzyme assay

The activity of extracellular amylase was followed by Bernfeld, 1955[9]**.** 1 mL of crude enzyme from each combination was taken in separate test tubes and 1 ml of 1% starch solution, 3.5 ml of citrate phosphate buffer (pH 7.0) was added to it. The reaction mixture was incubated at 40ºC for 30 min. 2 ml of DNS reagent was added to all the test tubes and then kept in boiling water for 5 minutes, then cooled. The amount of reducing sugars in the final mixture was quantified by DNS method according to Miller [10]. One unit (U/mL) of *α*-amylase activity is defined as: the amount of protein (*α*-amylase) required to liberate 1 μmol (0.18 mg equivalence) of reducing sugar (D-glucose) from starch/min, under the assay conditions.

2.6. Study of kinetic parameters of alpha amylase

Various kinetic parameters for fungal amylase were carried out such as effect of temperature, effect of pH, time of incubation and substrate concentration were studied.

2.6.1. Effect of temperature on amylase activity

Optimum temperature needed for amylase activity was estimated by incubating the reaction mixture at different temperatures from 4℃ -100℃ by dinitrosalicylic acid method

2.6.2 Effect of pH on amylase activity

The effect of pH on enzyme activity was studied by performing the enzyme assay at different pH using citrate phosphate buffer (pH range 3-11). The optimum pH of enzyme was determined by incubating the enzyme with different pH buffer as described above and assay was carried by dinitrosalicylic acid method**.**

2.6.3. Effect of incubation time on enzyme activity

The effect of incubation time on the activity of amylase was studied by performing the assay at different time intervals from 10 min-40 min.

2.6.4. Effect of substrate concentration on enzyme activity

Optimum concentration needed for enzyme activity was carried out by incubating the reaction mixture for 30 min at different concentration of starch solution (1%-4%) by dinitrosalicylic acid method**.**

3. RESULTS AND DICUSSION

3.1 Effect of different combination of agarowaste on amylase production

To check the agro-industrial wastes suitability for amylase production by *Aspergillus niger* sp was done with the addition of different combination of agrowaste as carbon source. Orange and papaya (2g:2g) was showed most suitable for amylase production (46U/ml)followed by orange and sweet lime (2g:2g)45.33U/ml and papaya and potato peel (2g:2g)38.33U/ml. Present study on utilization of agro-industrial wastes for the production of amylase showed that production of amylase is directly related to the raw starch content in agro-industrial wastes while high hemicellolose content in agro-industrial wastes have no effect on amylase production. This may be due to the preferred utilization of starch for their metabolic activities by *Aspergillus niger* over hemicellulose. It has been reported that production of extracellular enzymes (amylase and xylanase) on sugarcane bagasse as sole carbon source by *Thermomonospora curvata*.[11] Amylase production on gruel (wheat grinding by-product) based medium by *Aspergillus oryzae* was reported in SmF [12]. Many agro-industrial wastes (wheat bran, rice bran, yellow maize meal, dry yeast, brewery yeast and corn steep liquor) was used in basal medium as sole carbon source for the production of amylase by Streptomyces aureofaciens 77 in SmF. [13]

A lower yield of extracellular amylase production under SSF by *Aspergillus oryzae* using sugarcane bagasse has been reported [14]. High amylolytic activity of *Aspergillus niger* in biomass production found that *Aspergillus niger* was superior to other species of *Aspergillus niger* and

Table - 1: Effect of different combination of substrate concentration on amylase production by *Aspergillus niger*

Substrate combination in grams and amylase activity in U/ml/min			
Agrowaste Ratio in grams	**1g:3g**	**2g:2g**	**3g:1g**
Orange –Sweet lime	34.67	45.33	38.33
Orange –Papaya	40.33	46	36
Sweet lime –Banana	30.67	27.67	29.33
Orange –banana	34.33	34.5	32.3
Papaya sweet lime	30	26.33	28.33
Orange potato peel	32.67	35	34
Sweetlime potato peel	28.67	34.33	28.33
Papaya potato	35	38.33	33.67
Wheat bran sweet lime	27.5	22.33	20
Wheat bran papaya	21.67	26	25
Wheatbran sugar cane	24	23.33	19
Wheat bran orange	28.33	25.17	21.33

strains of fungi in biomass yield from agricultural waste [15,16]. Table 1 showed the quantitative assessment of the amylase productivity by using different combination of agarowaste in production medium.

3.2. Effect of temperature

For different incubation temperatures (4℃, 40℃, 70℃ and 100℃), maximum enzyme activity was observed at 70℃. When temperature was increased or decreased beyond this point, there was a decrease in enzyme activity (Figure 1).

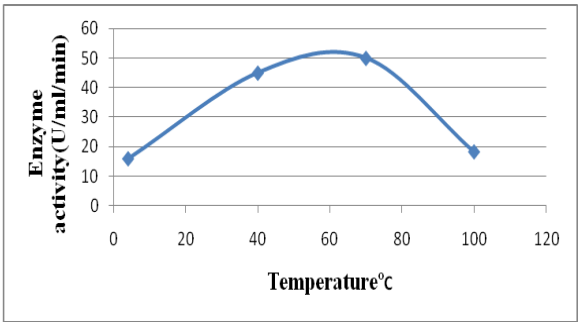

Figure - 1: Effect of temperature on enzyme activity.

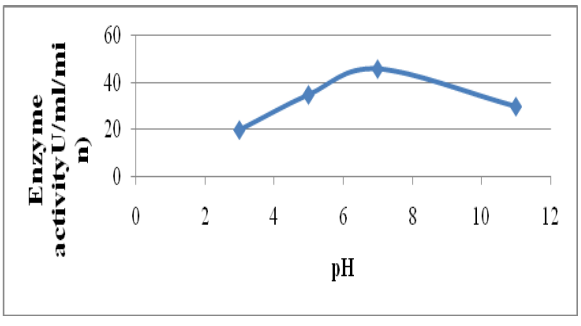

Figure - 2: Effect of pH on enzyme activity.

3.3. Effect of pH

For the selected *Aspergillus niger,* highest enzyme activity was observed at pH 7.0 As pH increased, enzyme production also increased with the highest value at pH 7.0. But when the pH was increased further, enzyme production was found to be decreased as indicated in figure 2.

3.4 Effect of incubation time

The optimum incubation time for fungal amylase found to be 30 min.

3.5 Effect of substrate concentration

Amylase activity increased on increasing starch concentrations (0.5%, 1.0%, 1.5% 2.0%, 2.5%, 3%, 3.5%, 4%). It was observed that enzyme activity increased linearly with the increase in substrate concentration. The optimum substrate concentration was found to be 3% as shown in figure 3.

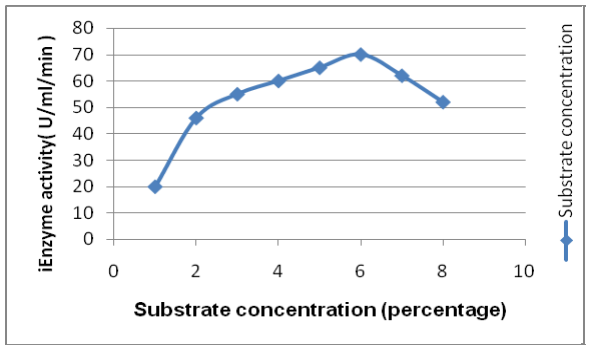

Figure - 3: Effect of substrate concentration on enzyme activity.

4. CONCLUSION

This study was focused on the isolation and selection of fungal strain which are capable of

producing industrially valuable extracellular amylase from the low cost carbon substrates to reduce the cost of enzyme production and make a better alternative for utilization of agrowaste. The use of low cost residues as substrates in enzyme production is especially interesting for countries where agro-industrial residues are abundant [17,18]. Amongst the various agro-industrial by-products, sugarcane bagasse and waste sisal are produced in large amounts. Recently, various researchers have utilized sugarcane bagasse for different purposes, *e.g.* as substrate for bioethanol and enzyme production [19,20].

Various agro industrial wastes like banana peel, orange peel, sweet lime peel, papaya peel, potato peel, wheat bran and sugarcane bagasses were used as substrate in the production media in different combinations instead of starch. All the combinations were found to be good substrates as amylase activity was seen in all the flasks. However, the maximum enzyme activity was in the flask containing 2g orange and 2g papaya as substrate and it was found to be 46U/ml/min followed by 2g orange and 2g sweet lime (45.33U/ml) and 2g papaya and 2g potato peel (38.33U/ml/min). It can be concluded based on the above study that combination of orange and papaya can be a very good substrate for the production of amylase when used in the ratio of 2:2. Other substrates combinations can also be used for industrial production of amylase but after proper optimization. pH study was carried out over the range of 3-11 pH and enzyme showed maximum activity at 7 pH which is slightly higher in case of agrowaste used as substrate.

For different incubation temperatures (4℃, 40℃, 70℃ and 100℃) were studied and highest enzyme activity was observed at 70℃. Different substrate concentrations from (0.5%, 1.0%, 1.5%, 2.0%, 3.0%, 3.5%, 4%)were used and it was observed that enzyme activity increased linearly with the increase in substrate concentration upto 3% after that it starts decreasing. Optimum time of incubation was carried out from 15-40 min and enzyme showed best activity when incubated for 30 min.

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