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PRODUCTION, PURIFICATION AND CHARACTERIZATION OF GLUCOAMYLASE BY SOLID-STATE FERMENTATION USING *ASPERGILLUS NIGER* WITH WHEAT BRAN AND RICE FLAKE AS SUBSTRATE

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ABSTRACT
Glucoamylase are amongst the main enzymes that are involved in the hydrolysis of starch. Glucoamylase produces high glucose syrup, and also used in fermentation processes for production of glucose and ethanol. In the current study, production of glucoamylase was done using wheat bran and rice flakes as substrate which is relatively very cheap source. The enzyme was produced by solid-state fermentation using Aspergillus niger. Initially fungal colonies were isolated from natural sources which were later tentatively identified as Aspergillus niger on the basis of colony characteristics and microscopic examination of mycelia. Fermentation for the production of enzyme was done using fermentation media inoculated with Aspergillus niger spores mixed with 0.0001% sodium lauryl sulphate. During fermentation O.D was measured and the activity was calculated every 24 h. Effect of fermentation duration, pH, temperature on enzyme was studied. The enzyme showed lowest activity of 0.77 U/gds at 24 h and highest activity of 1.29 U/gds at 96h of fermentation duration. Enzyme showed highest activity of 1.86 U/gds at 5.2 pH and lowest activity of 0.68 U/gds at 3.6 pH. Similarly, the enzyme activity was highest i.e. 2.25 U/gds at 55°C and was lowest i.e. 0.68 U/gds at 90°C. Finally, the glucoamylase produced was purified for further use and the enzyme activity was calculated.
Keywords: *Aspergillus niger, fermentation, glucoamylase, enzyme activity, wheat bran, rice flakes.*

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INTRODUCTION

Amylases are the enzymes that catalyse the hydrolysis of α-1,4 glycosidic bonds and produces simple sugars i.e. glucose. It also carries out the hydrolysis α-1,6 glycosidic bonds of polysaccharides forming end product glucose.

Amylases are responsible for digestion of starch. Amylase enzymes are applied in many processed food business such as food fermentation and also in pharmaceutical industries [1]. Amylases produced using microorganisms such as bacteria, yeast and molds is quite a cheap process as it uses very cheap source for substrate is employed.

Along with hydrolysis of starch glucoamylases are applied for production of high glucose syrup, and also in fermentation processes for production of beer and ethanol. Enzymes are produced by various fermentation processes like solid-state and submerged fermentation. Solid-state fermentation gives better result as compared to submerged fermentation for production of glucoamylase. The primary requirement to carry out a fermentation process is a potential organism. In industries, organisms such as *Aspergillus oryzae, Aspergillus niger, and Rhizopus oryzae* are used on large scale. Wheat bran and soybean serve as vital substrate for fermentation process. The glucoamylase produced by fungal species are quite stable in nature. When we carry out fermentation of microorganisms we need to maintain temperature, pH for obtaining proper yield. The duration of fermentation also affects the production of enzyme.

MATERIAL AND METHODS

Collection of samples:

Soil sample was collected from nearby area of Malkapur, Karad.

A] Isolation of fungi from soil:[1][12]

1g of soil sample was serially diluted and spread inoculated on Sabourauds agar plates. These plates were incubated at 30°C for 72 h-96 h.

Identification of fungus:[1][12]
Identification of the fungus was done colony characteristics and microscopic examination of mycelia and reproductive structures.
Preservation of fungus: [4]
Isolated fungus was transferred on Sabourauds sporulation medium. It was incubated aerobically at 30°C for 72 h and was subcultured 3 to 4 weeks and preserved at 4°C.
B]Inoculum preparation:[3]
The isolated culture of *Aspergillus niger* was taken from laboratory. 1 mL of inoculum having 10^6 spores per mL collected from 72 h grown culture of *Aspergillus niger*. Here, well isolated representative colonies were picked up and transferred into inoculum media contained 5 g of solid substrate and 10 mL mineral salt solution containing (mg/gds) K_2HPO_4, KH_2PO_4, NaCl, $CaCl_2.2H_2O$, Boric acid, $(NH_4)_2SO$, $MgSO_4.7H_2O$, $CuSO_4.5H_2O$, $ZnSO_4.7H_2O$, $MnSO_4.H_2O$, $FeSO_4.7H_2O$ in the 100 mL flask and this flask was incubated at room temperature for 24-48 h.
C] Production media preparation:[3]
Production of glucoamylase was carried out in a 125 mL fermentation medium with 3 g of dry substrate inoculum of *Aspergillus niger* mixed with sodium lauryl sulphate(0.0001%) and aseptically inoculated into fermentation media. The flask was incubated at 28-30°C and enzyme production was checked after every 24 h for 5 days.
D] Production of Glucoamylase:[3][10]
After completion of fermentation, enzyme was extracted in 200 mL, 0.1M sodium acetate buffer and pH was adjusted and was kept on a rotary shaker at 250 rpm for 30 min. The content was filtered through muslin cloth and filtrate was used as enzyme source. The effect of fermentation duration was studied at 0, 2h, 48h, 72h, 96h, 120h and the duration at which maximum yield of enzyme was obtained was taken as optimal incubation period of fermentation.
E] Purification of Glucoamylase:[3][10]
The crude extract was subjected to partial purification by ammonium sulphate precipitation and was subjected to dialysis.
a)Ammonium sulphate precipitation:[9]
The supernatant was fractionated by precipitation with ammonium sulphate of 50% of saturation. All subsequent steps were carried out at 4°C. The protein was resuspended in 0.1M Phosphate buffer at pH 7 and dialyzed against distilled water.
b)Dialysis:[9]
The resuspended protein in 0.1M phosphate buffer was taken inside a dialysis bag and bag should be half filled and two ends are tightly packed to prevent leakage. Bag is now submerged in beaker containing about 100 mL of distilled water and the contents are kept at 4°C. Salt molecule passes out freely. Repeat the process twice for reducing salt concentration inside the bag to negligible level. Water being fully permeable enter into bag and dilutes the protein. Finally, enzyme can be purified. Enzyme was assayed using DNSA method. The effect of temperature and pH on enzyme activity was also studied.
Yield of glucoamylase:
One unit(U) of glucoamylase activity is defined as the amount of enzyme that releases one micromole of reducing sugar as a glucose, per minute, under assay conditions and expressed as U/g of dry substrate(gds)
Formula:
1 unit of glucoamylase was calculated by using formula,

$$\frac{\mu g \text{ glucose} \times \text{total volume of reaction mixture(mL)} \times \text{total volume of buffer added}}{180.01 \times \text{enzyme used(mL)} \times \text{Incubation time(min)} \times \text{Initial dry wt. of substrate}}$$

RESULTS AND DISCUSSION

Table-1Cultural and morphological characteristics of fungal isolate:

Morphological characters	Observation
Shape	Circular
Colony size	3 cm
Sporulation	Aerial
Spore color	Black
Mycelial properties	Coumella spores in chain and foot cells was observed.

The morphological characteristics of the isolate were studied. The observations are listed in the Table-1.

Table-2 Effect of fermentation period on glucoamylase enzyme production:

Sr. No.	Fermentation duration period in hours	O.D. at 530 nm(in enzyme assay)	Activity in U/gds
1	24	0.32	0.77
2	48	0.35	0.90
3	72	0.49	1.03
4	96	0.52	1.29
5	120	0.37	0.95
6	Black	0.0	0.0

Effect of fermentation duration on the enzyme was studied. It was recorded that enzyme shows highest activity at 96 h of fermentation and further reduction occurred and lowest activity was recorded at 24 h.

Fig-1 Effect of fermentation duration on enzyme activity:

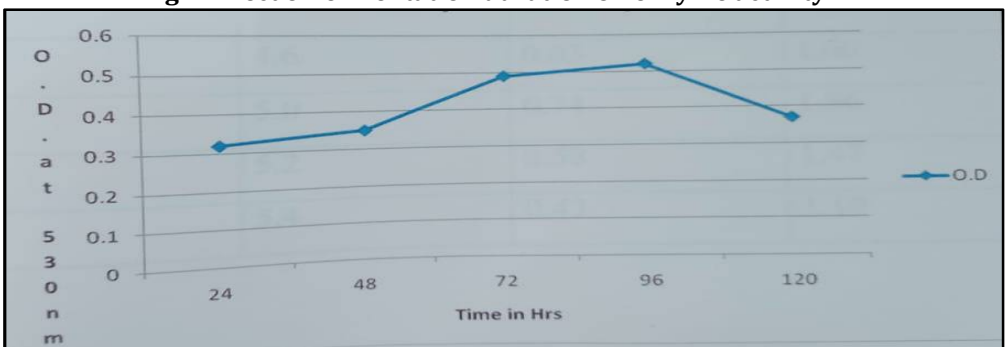


Table-3 Effect of pH on enzyme activity:

Sr. No.	pH of medium	O.D. at 530 nm(in enzyme assay)	Enzyme activity in U/gds
1	3.6	0.47	0.68
2	4.6	0.63	1.60
3	5.0	0.74	1.86
4	5.2	0.58	1.47
5	5.4	0.43	1.10

Effect of pH on enzyme production was studied. It was recorded that enzyme showed highest activity at pH 5.0 and lowest activity at pH 4.6.

Fig-2 Effect of pH on enzyme activity:

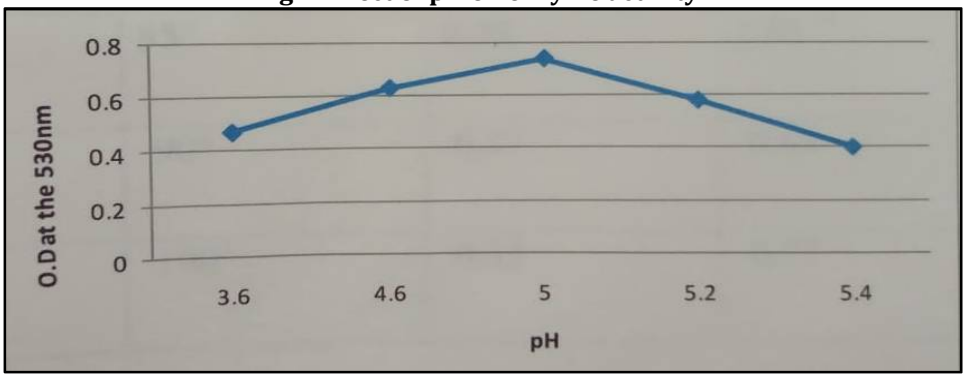
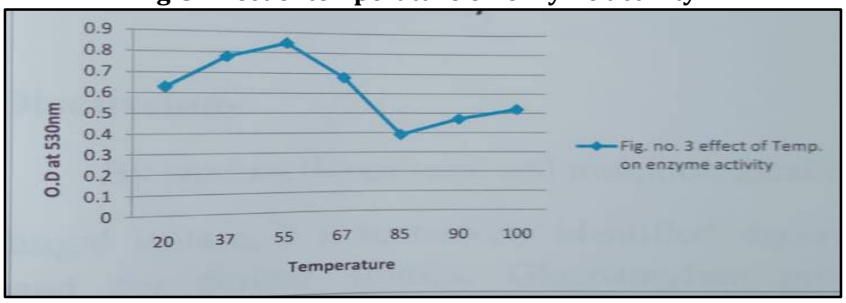


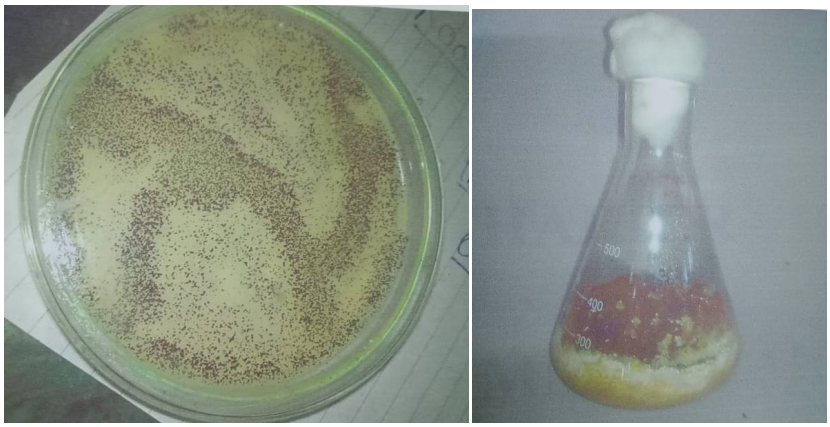
Table-4 Effect of different temperature on enzyme activity:

Sr. No.	Tube type(Temp)	O.D. at 530 nm	Enzyme activity in U/gds
1	20°	0.63	1.60
2	37°	0.78	1.97
3	55°	0.85	2.25
4	67°	0.68	1.73
5	85°	0.39	1.01
6	90°	0.47	0.68
7	100°	0.32	0.77

Effect of different temperatures on enzyme activity was studied. It was recorded that enzyme showed highest activity at 55°C and lowest activity at 90°C.

Fig-3 Effect of temperature on enzyme activity:





1 **2**

Photoplate-1 Isolates at 30°C after 96h incubation on Sabouraud's agar:
Photoplate 2) Glucoamylase production medium (surface culture technique):

DISCUSSION

The isolate which was obtained was tentatively identified as *Aspergillus niger* on the basis of morphological and cultural characteristics. Glucoamylase produced by Aspergillus niger is a very important enzyme used in enzymatic degradation of starch to produce high fructose corn syrup. Arasaratnam *et al*.,[2] have reported glucoamylase production by *Aspergillus niger* using rice flake and wheat bran as a waste substrate. As discussed earlier glucoamylase activity was optimum at 55°Cand at pH 5 when it was carried out at different temperature and pH using 1% soluble starch as substrate for enzyme assay.

CONCLUSION

Glucoamylase produced by the fungus *Aspergillus niger* is a very important industrially and used in the enzymatic degradation of starch. Glucoamylase was isolated from the biomass of*Aspergillus niger.* The results provided valuable information for the production of Glucoamylase by *Aspergillus niger* using relatively very cheap substrate i.e. wheat bran and rice flake.

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CONFLICT OF INTEREST: No conflict of interest is there amongst authors.

REFERENCES

1. Adefisoye SA, Sakariyau AO. (2018). Production of Glucoamylase by Aspergillus niger in Solid State Fermentation. Adv. Biol. Res. 2:7-11.
2. Alexopoulos CJ. (1962). Introductory mycology. Introductory mycology.3rd Edition:pp490
3. Casida LE. (1968). Industrial microbiology. Industrial microbiology.pp87
4. Cruickshank AM. (1985). Gordon Research Conferences: 1986 Winter Schedule. Science. 230(4722):189-95.
5. Deshmukh AM. (1987). Handbook of Media. Stains and Reagents in Microbiology Pama Publication.
6. Jayaraman J, Jayaraman J. (1981). Laboratory manual in biochemistry. Delhi, India:: Wiley Eastern.
7. Kulkarni GT. (2002). Biotechnology and its applications in pharmacy. Jaypee Bros Medical Publishers.
8. Lehninger AL. The molecular basis of cell structure and function. Biochemistry. 1975:220.
9. Mu P, Plummer DT. (2001). Introduction to practical biochemistry. Tata McGraw-Hill Education.
10. Peppler HJ, Perlman D, editors. (2014). Microbial technology: Fermentation technology. Academic Press.
11. Purohit SS, Kakrani HN, Saluja AK. Pharmaceutical biotechnology. Agrobios (India); 2003.
12. Sutton BC. (1980). The Coelomycetes. Fungi imperfect with pycnidia, acervuli and stromata. Commonwealth Mycological Institute.1110
13. Scale M. (2009). Wikipedia the Free Encyclopedia.

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