International Journal of Advances in Engineering and Management (IJAEM)
Volume 5, Issue 6 June 2023, pp: 579-582 www.ijaem.net ISSN: 2395-5252

Studies on Production and optimization of novel α-amylase by using wheat bran residue from Streptomyces enissocaesilis under solid state fermentation

Balatejeswara Rao V, Praveen Krishna V
Centre for Biotechnology, Department of Chemical Engineering, College of Engineering, Andhra University, Visakhapatnam, Andhra Pradesh, India.

Date of Submission: 10-06-2023 Date of Acceptance: 20-06-2023

ABSTRACT
Solid state fermentation was carried outusing various agro-industrial wastes with the best amylase producing strain isolated from marine. Different physiochemical conditions were varied for maximum enzyme production. The strain produced about 30.22±0.9 U/gds of amylase at 60% moisture content, 20% inoculum, after 120 h of incubation with wheat bran as the substrate. The optimum temperature and pH of the enzyme activity were found to be 32°C to 7 respectively.
Keywords: Solid State Fermentation, amylase, Marine actinomycete.

I. INTRODUCTION

Amylase is one of the most widely used enzymes in industry. It hydrolyses starch and is used commercially for the production of sugar syrup from starch which consists of glucose, maltose, and higher oligosaccharides. Amylases are of great significance in biotechnological applications ranging from food, fermentation, detergent, pharmaceutical, brewing and textile to paper industries. To meet higher demand of these Industries low cost production of amylase is required.

Amylase is produced in bacteria, fungi, plants and animals. However, due to efficient production strategies microorganisms have substantial potential to contribute to a number of industrial applications. Such industrially important microorganisms are found within the actinomycetes species because of their rapid growth rates that lead to short fermentation cycles, their capacity to secrete proteins in to extracellular medium, and general handling safety.

Production of these α-amylases has been investigated through submerge (SmF) and solid-state fermentation (SSF). However, the contents of a synthetic medium are very expensive and economical, so they need to be replaced with more economically available agricultural and industrial by product as they are considered to be good substrates for SSF to produce enzymes. In recent years the technique of solids-state fermentation (SSF) process has been developed and used more extensively. It has advantages over SmF like simple technique, low capital investment, cheaper production of enzyme having better physiochemical properties lower levels of catabolite repression and better product recovery. The major factors that affect microbial synthesis of enzymes in a SSF system include selection of a suitable substrate and microorganism, particle size of the subset.Inoculum concentration and moisture levels of substrate. Thus it involves the screening of a number of Agro industrial materials for microbial growth and product formation. Temperature and pH are known to be important parameters in the production of enzymes from actinobacteria. The present work represents an investigation into amylase production by SSF with Wheat bran as substrateand the determination of optimized production conditions.

II. MATERIALS AND METHODS

Isolation of actinomycetes

About 21 strains were isolated from marine samplefromBay of Bengal(Visakhapatnam, India) were collected. Thesestrains were characterized by colony morphological shape and gram character and maintained in stock culture till further use.

Screening of bacterial isolates

Primary screening of actinomycete lab isolate for production of α-amylase was done by the starch Casein Agar plate method out of 21

International Journal of Advances in Engineering and Management (IJAEM)
Volume 5, Issue 6 June 2023, pp: 579-582 www.ijaem.net ISSN: 2395-5252

strains, the 6 strains that showed the biggest zone of clearance in starch hydrolysis were a selected for production in solid fermentation.

Substrates

Different types of Agro Industrial residues to be used as a substrate.Wheat branresidue procured from local market of Visakhapatnam and powdered obtains in a particle size. SSF was performed with all the four substrates and their enzyme production was checked by assay.

SSF Technique

Experiments were conducted in 250ml Erlenmeyer flasks containing 5 grams of substrate impregnated with moisture content. The flasks were autoclaved and inoculated with 1ml ofthe prepared inoculum. Thoroughly mixed and followed by incubation at 32°C for 6 days. Samples were aseptically withdrawn periodically and assayed for amylase activity.

Enzyme assay

Estimation of amylase activity was carried out according to the DNSA (3,5 dinitro salicylic acid) method. 1 ml of 1% starch was incubated with different dilutions of the enzyme extract. The reaction mixture was incubated at 32°C for 30 min. The reactionmixture was stopped by adding to 1 ml of DNS and kept in boiling water bath for 10 min. The absorbance was read at 540nm using a Spectrophotometer, against glucose as the standard.One unit of enzyme activity is defined as the amount of enzyme, which releases 1 µmole of reducing sugar as glucose per minute under the assay conditions (U/ml). The experiments were carried out in triplicates and standard error was calculated. In a sequential order, the various physicochemical factors as substrate, moisture, inoculum size affecting the enzyme production were optimized for maximal enzyme production by using the solid substrate for which best amylase activity was observed.

$$\text{Amylase activity (U/ml)} = \frac{(\text{U moles of glucose released})(\text{total reaction mixture})}{(\text{Time of incubation})(\text{mg of enzyme in reaction mixture})}$$

$$\text{Amylase activity (U/gds)} = \frac{(\text{Units/ml enzyme})}{\text{Grams of dry soil/ ml enzyme}}$$

Optimum time

Fermentation time for the enzyme assay from 1 to 6 days enzyme activity was increases with optimum time of incubation.

Optimum temperature

Optimum temperature for the enzyme assay was assessed by varying the incubation temperature of the assay from 28°C to 36°C.

Optimum pH

Optimum pH for the enzyme assay was assessed by performing the assay with buffers ranging from pH 5 to 9 (citrate phosphate buffer for pH 5-8 & Tris-HCl buffer for pH 9).

Moisture content

Moisture content for the solid state fermentation of the enzyme was evaluated by varying moisture content from 20%, 40%, 60%, 80% and 100%.

III. RESULTS AND DISCUSSIONS

Six strains were selected on the basis of the zone of clearance they exhibited in the starch test, for the SSF technique. Fig 1 shows the enzyme production by these strains in utilizing Wheat bran as substrate in the solid state fermentation.

Strain showed the maximum activity 11.09 U/gds. The production in all the six strains increased till day 5, where they showed the maximum enzyme activity, thereafter, decreasing substantially till day 6. Strain, a gram positive bacterium, was selected for further testing and optimization. Fig 2, Fig 3, Fig 4 and Fig 5shows the best enzyme production of different optimization parameters like time 5^{th} day, temperature 32°C, pH 7, moisture content 20% and inoculum size 20% ratios respectively found the enzyme production was30.22±1.2 U/gds.

Isolated the strain exhibited a large zone of clearance in starch test which showed that it was able to produce amylase in a substantial quantity. From the industrial viewpoint it is necessary to have a strain that can produce a large quantity of the enzyme in short fermentation time. Our strain produced very high amount of amylase.

Moisture content is a critical factor for SSF processes because this variable as influence and growth and biosynthesis and secretion of different metabolites. Lower moisture content causes reduction in solubility of the nutrients of the substrate, low degree of swelling and higher water tension. Higher moisture levels can cause a reduction in enzyme yield due to steric hindrance

International Journal of Advances in Engineering and Management (IJAEM)
Volume 5, Issue 6 June 2023, pp: 579-582 www.ijaem.net ISSN: 2395-5252

of the growth of strain by reduction porosity of the solid substrate,

Inoculum size is an important factor for the production of enzyme. Lower inoculum level results in a lower number of cells in the production medium. This requires a longer time to grow to an optimum number to utilize the substrate and form the desired product. It is evident from this study that wheat Bran Agro waste may serve as ideal fermentation bases for obtaining high yields of amylase from Streptomyces enissocaesilis.

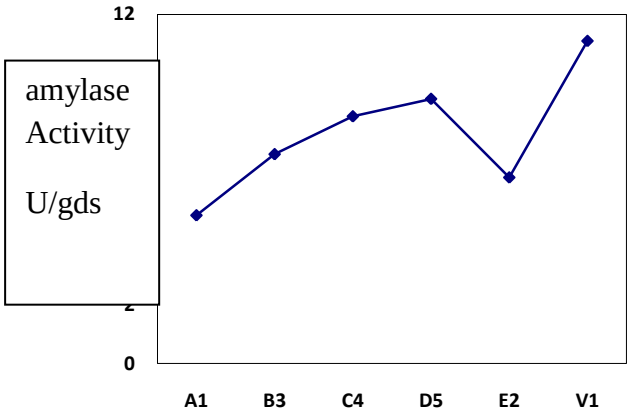


Fig 1: amylase production by six strains by using wheat bran residue as a substrate V1 shows highest activity on 5th day.

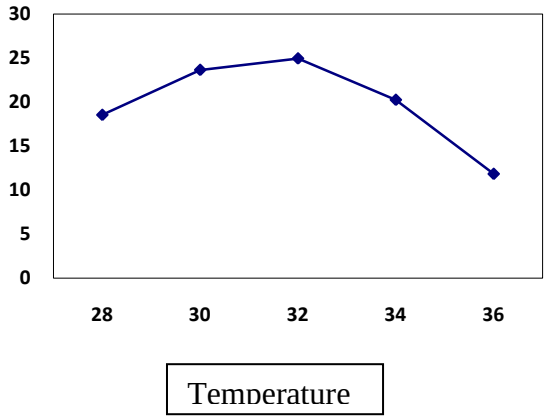


Fig:2

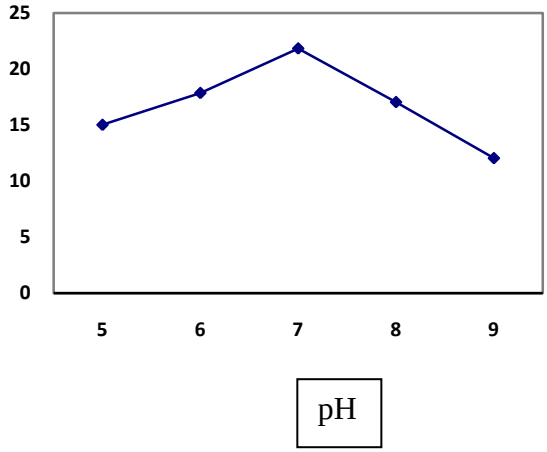
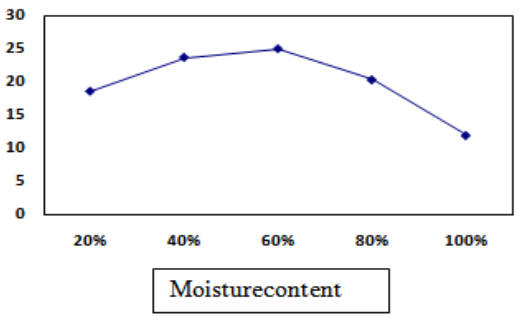
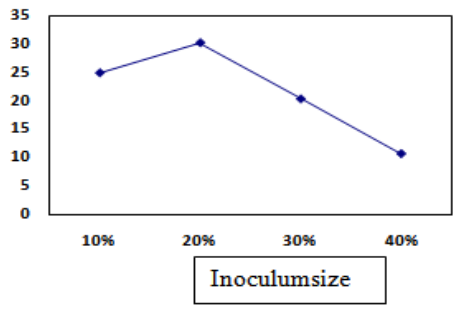


Fig:3





X-axis indicate optimization parameter, Y-axis indicate enzyme activity U/gds.

IV. CONCLUSION

From the present findings, it can be concluded that Indian Ocean is a potential source for wide spectrum of bioactive metabolite producing actinomycetes. Only a few investigations carried out on enzyme production by actinomycetes isolated from marine sediments have been published.

According to the findings of this study, marine sediments include actinomycetes that synthesize industrially essential extracellular enzymes. They could be used as one of the potential sources for biotechnological applications in various of fields.

REFERENCES

[1]. Adinarayana Kunamneni., Kungen Perumal., Suren Singh. Journal of Bioscience and Bioengineering 2005; 100(2):168-171.

[2]. Chakraborty S, Khopade A, Biao R, Jian W, Liu XY, Mahadik K, & Kokare C. Characterization and stability studies on surfactant, detergent, and oxidantstableα-amylase from marine haloalkaliphilic Saccharopolyspora sp. A9. Journal of Molecular Catalysis B: Enzymatic. 2011; 68(1):52-58.

[3]. EL-Banna TE, Abd-Aziz AA, Abou-Dobara MI, and Ibrahim RI. Production and immobilisation of -amylase from Bacillus subtilis Pakistan J. of Biol. Sci. 2007; 12:2039–2047.

[4]. Gupta R, Gigras P, Mohapatra H, Goswami VK & Chauhan B.Microbial α-amylase:A biotechnologicalperspective. Proc.Biochem. 2003; 38:1599-1616.

[5]. P. Ellaiah and A. P. C. Reddy, "Isolation of Actinomycetes from Marine Sediments off Visakhapatnam, East Coast of India. 2008; IJMS Vol.16(2).

[6]. Poornima R, Sahu MK, Sivakumar K, & Pushpavalli V. Optimisation of -amylase production by Actinomycete strain AE-19 isolated from shrimp pond. Trends Appl. Sci. Res. 2008; 3: 45–52.

[7]. Ragunathan R. and Padhmadas R. Production, purification, and characterization of -amylase using Streptomyces spp. PDS1 and Rhodococcus spp. Isolated from the Western Ghats Int. J. Curr. Microbiol. App. Sci.2013; 2(8):206–14.

[8]. Rajshree Saxena., Rajni Singh. Amylase production by solid-state fermentation of agro- industrial wastes using Bacillus sp. Braz J microbial.2011;42(4): 1334-1342.

[9]. Renu Singh, Vishal Kapoor, and Vijay Kumar PRM utilised agroindustrial wastes for the simultaneous production of amylase and xylanase by thermophilic actinomycete solid-state fermentation. Brazilian Journal of Microbiology. 2013; 1545–1552.

[10]. Samrat Chakraborty., Abhijit Khopade., Chandrakant Kokare., Kakasaheb Mahadik., Balasaheb Chopade. Isolation and characterization of novel α-amylase from marine Streptomyces sp.D1. Journal of Molecular Catalyssis B: Enzymatic. 2009; 58:17-23.

[11]. Swain MR and Ray RC. Alpha amylase production by Bacillus subtilis CM3 in solid-state fermentation using cassava fibrous residue. J. Basic Microbiol. 2007; 47: 417–425.

[12]. Saxena KR, Dutt K, Agarwal L, and Nayyar P. A highly and thermostable alkaline amylase from Bacillus sp. PN5. Bioresour. Technol.2007; 98:260–265.

[13]. Umadevi T. and Ragunathan R. Extracellular Production of Amylase and Xylanase from Streptomyces mcasbt1 Isolated from Marine Mangrove Sediment International Journals of Biotechnology and Biochemistry. 2013; [S.l.], 23–30.

[14]. Xu Dong Liu., Yan Xu. A novel raw starch digesting α-amylase from a newly isolatedBacillus sp. YX-1: Purification and characterization. Bioresource Technology 2008; 99: 4315-4320.

[15]. Yang SS and Wang JY. Protease and amylase production of Streptomyces submerged and solid-state cultivations. Bot. Bull. Acad. Sin.1999; 40, 259–265.