EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 3
Issue: 5
516-518
Year: 2016

BIOPROCESSING OF POMEGRANATE PEEL WASTE FOR α - AMYLASE PRODUCTION BY *BACILLUS SUBTILIS*.

Pooja V. Deshmukh, Kiran Damodar, Nazia D. Khan and Zia H. Khan*

Department of Biochemistry, Shri Shivaji College of Arts, Commerce and Science, Akola (M.S.)

***Author for Correspondence: Dr. Ziaulhasan Khan**
Department of Biochemistry, Shri Shivaji College of Arts, Commerce and Science, Akola (M.S.).

Article Received on 06/03/2016 **Article Revised on 30/03/2016** **Article Accepted on 19/04/2016**

ABSTRACT
α-amylase was produced by *Bacillus subtillis* utilizing pomegranate peel waste in a solid state fermentation (SSF). Enzyme production parameter was optimised by varying different carbon source concentration, Incubation period, pH and temperature. 20gm of Pomegranate peel waste in the inoculum media yielded maximum enzyme production. The maximum activity of α-amylase (21.87IU/min) was recorded after 72 hours of SSF at pH 7 and 35°C temperature of the optimum fruit waste medium. The study suggest that Pomegranate peel waste could be used as a potential raw material for alpha amylase production.

KEYWORDS: *α amylase, Bacillus subtillis, Pomegranate peel waste.*

INTRODUCTION
α-amylase is a hydrolytic enzyme and in recent year, interest in its microbial production has increased dramatically due to its wide spread use in food, textile, baking and detergent industries (Asghar et. al. 2000, 2002). Besides its use in saccharification and liquefaction of starch, the enzyme is also use for the warp sizing of textile fibres, clarification of haze formed in bear and fruit juice and for the pre-treatment of the animal feed to improve the digestibility (Stedt, 1988).

Amylases were one of the first enzymes to be produced commercially by microorganisms. Amylases are amylolytic enzymes; represents a group of catalytic proteins of great importance in carbohydrate metabolism (M. Nagajan *et al*., 2010). This Paper reports the optimisation of fermentation parameters for α amylase production by Bacillus Subtillis through SSF of Pomegranate peel waste.

MATERIAL AND METHODS
Pure culture of *Bacillus subtillis* was obtained from the Department of Microbiology of Shri Shivaji College, Akola, which was maintained at nutrient agar starch at pH 7 and 35℃ temperature, in the present investigation pomegranate peel waste was used as a carbon source and it was obtained from the market of Akola city.

Preparation of Substrate
Pomegranate peel waste was spread on the roof and sun dried for 48 hrs. After sun drying the source of substrate was collected and oven dried at 70°C for 24 hrs. Oven dried source of substrate was grounded to powder in an Electric grinder. The above grinded substrate taken in 250 ml conical flask was moistened with salt solution containing gm / 100 ml : yeast extract 0.3, peptone 0.5, NaCl 1.5, $Na_2HPO_4.2H_2O$ 1.1, NaH_2PO_4 0.61, KCl 0.3 and $MgSO_4.7H_2O$ 0.01. The substrate was moistened to 150% (W/V) by salt solution. Moistened substrate was taken in to autoclave and sterilized for 15 minute at 121°C for proper cooking of the substrate and to increase its amenability for microorganisms.

Inoculum Preparation
Bacillus Subtillis spores were transferred aseptically to 100 ml conical flask containing 50 ml of sterilized inoculum medium (sterilized at 12°C for 15 minutes) containing g/100ml: glucose 2, yeast extract 0.3, peptone 0.5, NaCl 1.5,$Na_2HPO_4.2H_2O$ 1.1, NaH_2PO_4 0.61, KCl 0.3 and $MgSO_4.7H_2O$ 0.01 in laminar air flow. The flask was then kept in incubator at 37°C for 48 hrs.

Solid State fermentation
After sterilizing, the substrate was cooled to room temperature. Substrate of 15 gm in conical flask of 250 ml was added with the inoculum of 30 % (W/V) in the laminar air flow with the help of sterilized pipette. *Bacillus Subtillis* was inoculated on pomegranate peel waste. After inoculation the flask were incubated at 37°C for 2 days. The SSF media flasks were gently shaken after every 12 hrs for uniform mixing of the substrate and microorganism. Growth medium containing pomegranate peel waste were incubated for (24-96 hr) at pH 7 and 35°C. Conical flask containing different carbon

source level (5-25 gm) were inoculated in (25 ml) and incubated for 72 hr at pH 7 and 35°C temperature. pH of 10 gm of pomegranate peel waste was adjusted at different levels (4, 5, 6, 7and 8) before inoculation and incubation for 72 hrs. Pomegranate peel waste (10 gm) were inoculated (25 ml) and incubated at pH 7 under different temperature viz 25, 30, 35 and 40 C for 72 hrs. (Nazia Khan & Zia Khan, 2009)

Enzyme Extraction

After incubation, the fermented pomegranate peel waste sample was mixed with 0.1 M sodium phosphate buffer (ratio 1:10 (w: v)) of pH 6.9 in to each conical flask. The fermented substrate was taken in 250 ml conical flask in laminar air flow and then buffer was added. The flask was shaken at 150 rpm for 60 minute and material was filtered through muslin cloth or was filtered through whatmann filter paper 1. Filtrate collected was centrifuged at 1000 rpm for 10 minutes at room temperature. Supernatant was carefully collected and used as crude enzyme extract for determining amylase activity.

Enzyme Assay

Amylase enzyme activity was estimated by DNSA (Di-Nitro Salicylic Acid) method. The reducing sugars produced by the action of α and β amylases reacts with dinitro salicylic acid and reduce it to a brown coloured product dinitro amino salicylic acid.

RESULTS AND DISCUSSION

Different fermentation parameters were optimized for the α amylase production by conducting a series of experiment and the result have been discussed as under. Duplicate flask containing 10 gm of pomegranate peel waste autoclave and incubated for 24, 48, 72 and 96h the maximum activity of α amylase (0.24 mg/ml maltose)was found after 72 hour of SSF at pH 7 and 35°C temperature. Similarly Baipai *et al.*, (1992) had produced α amylase by B. Licheniformis 44 MG 82-G, using glucose as a carbon source and optimum enzyme activity of culture. Medium was recorded after 96h. Fermentation media containing 5, 10, 15, 20 and 25 gm of pomegranate peel waste were sterilized, inoculated and incubated for at pH 7 and 35°C. It was observed 20 gm of pomegranate peel waste in the fermentation medium yielding maximum (0.5 mg/ml maltose) (fig.1) α amylase after 72 hour at pH 7 and 35°C. A further increase dose not increased in substrate level. Krishna and Chandrasekaran (1996) reported maximum alpha amylase producing by B.subtilis utilizing 10 gm of banana stalk as substrate. Data showing the effect of pH on α amylase production by B. Subtilis in SSF of pomegranate peel waste are presented in fig 2. The maximum activity of alpha amylase (0.25 mg/ml maltose) was observed in the fermentation medium adjust at pH 7.At pH 4, activity was law due to more acid but as the pH increase to 7 the enzyme induction increase our result are similar to those of Terui (1973), who reported 6.8 as optimum pH for the production of alpha amylase by B. subtilis. Bacillus subtilis showed maximum activity of alpha amylase (0.25 mg/ml maltose)the pomegranate peel waste medium (10gm)when incubated at 35°C for 72 h at pH 7.A decrease or increase in temperature caused decrease in the enzyme production by B.subtilis fig 3. The optimum temperature observed for alpha amylase production from banana stalk *B.subtilis* was also 35°C as reported by the Krishna and Chandrasekaran (1996).

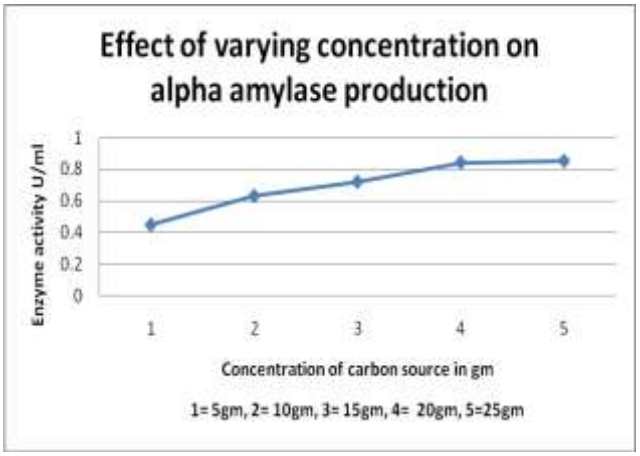

Figure. 1

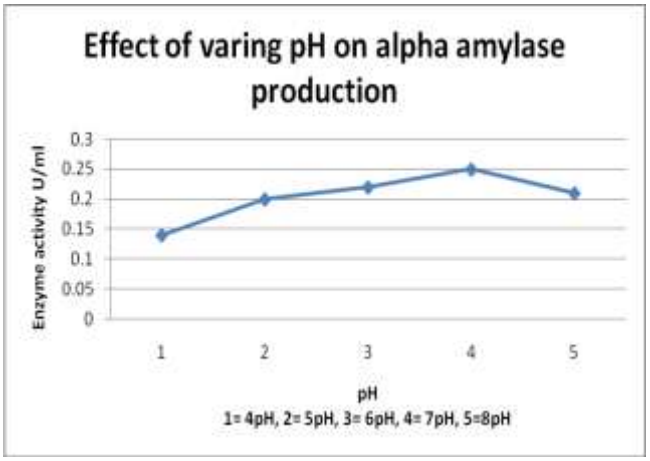

Figure. 2

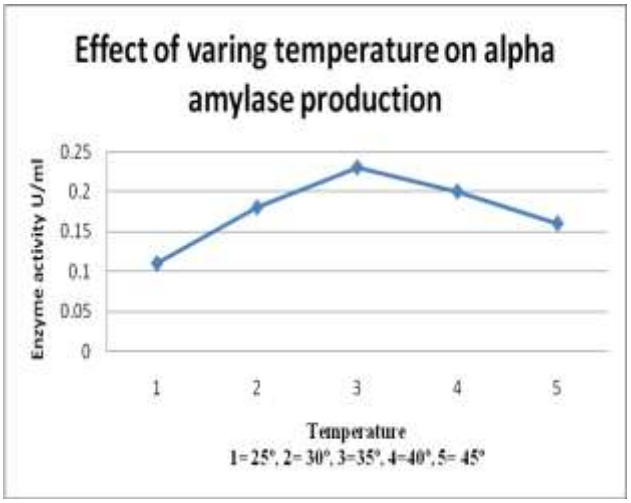

Figure. 3

REFERENCES

1. Asghar M. M. S. Rafiq, U. Azhar and MM. Asad,: Production of alpha amylase by Archniotus Sp. Using waste bread medium. *Int. J. Agri. Biol*., 2002; 4: 26-28.
2. Bajpai P., P. K. Gera and P. K. Bajpai,: Optimization studies for the production of alpha amylase using cheese whey medium. *Enzyme and Microbial Technol*., 1992; 14: 679-83.
3. Henes, B. and S. Stedt,: Milling and baking technology, PP 415-70, John Wiley and Sons, USA, 1988.
4. Krishna, C. and M. Chandrasekaran,: Banana waste as substrate for alpha amylase production by Bacillus subtillis under solid state fermentation. *Appl. Microbial Biotechnol*, 1996; 46: 106 - 11.
5. Nagajan, M., T. Deborah Paripuranam and Umamaheshwari, S.. Efficient production of alpha amylase from agro residues using *Bacillus subtilis. J.Chem. Pharma. Res*., 2010; 2(4): 442-448.
6. Nazia D. Khan and Zia H. Khan : Bioprocessing of Sugar cane waste for alpha amylase production by Bacillus subtilis. *Biosci. Biotech. Res.Comm*., 2009; 2: 220-222.
7. Sharanappa A., K. S. Wani., Pallavi Patil: Bioprocessing of Food industrial waste for alpha amylase production by solid state fermentation. *International Journal of Advanced Biotechnology and Research*, 2011; 2(4): 473 - 480.
8. Terui G.: Kinetics of hydrolyses production by microorganism. In sterhackk (Ed), Microbial Engineering, 2nd Ed., PP: 377-95, Butterworth, London., 1973.