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BIOPROCESSING OF FOOD INDUSTRIAL WASTE FOR α-AMYLASE PRODUCTION BY SOLID STATE FERMENTATION

Sharanappa A[1]., K.S.Wani[2]., Pallavi Patil[3]
[1,3]Department of Biotechnology, KIT's COE, Kolhapur,
[2]Department of Chemical Engineering, SSBT's C.O.E.T., Bambhori, Jalgaon.

ABSTRACT

α-amylase was produced by *Aspergillus niger* utilizing fruit waste in a solid state fermentation (SSF). The effect of varying particle size, extraction pH, incubation pH, incubation temperature, activity temperature, incubation period, moisture content, peptone and yeast extract content on the production of α-amylase was investigated. The maximum activity of α-amylase (21.87IU/min) was recorded after 48 hours of SSF at pH 6 and 25^{o}C temperature of the optimum fruit waste medium. The enzyme produced by *Aspergillus niger* can be used in industrial processes after characterization.

Key words: Fruit Waste, α-amylase, *Aspergillus niger,* Solid state fermentation.

[I] INTRODUCTION

Alpha amylase is a hydrolytic enzyme and in recent years, interest in its microbial production has increased dramatically due to its wide spread use in food, textile, baking and detergent industries [1]. Besides its use in the saccharification or liquefaction of starch, the enzyme is also used for the warp sizing of textile fibres, the clarification of haze formed in beer or fruit juices, and for the pretreatment of animal feed to improve the digestibility. A growing new area of application of α-amylase is in the fields of laundry and dish washing detergents [18]. Processing of fruits like mango, banana, papaya and citrus fruits for production of pulp produces huge solid waste in form of peel, skin, seeds, stones etc is either discarded in soil or used as animal feed. In some fruits the discarded portion can be very high(eg mango 30-40%, banana, papaya 20% and orange 30-50%).This waste often causes a serious waste disposal problem, which can lead to problem with flies and rats around the processing room if not correctly dealt with.

Since fruits are rich in carbohydrates and other nutrients, it can serve as substrate for α-amylase production by solid state fermentation. This paper reports the optimization of fermentation parameters for α-amylase production by *Aspergillus niger* through solid state fermentation (SSF) of fruit waste for possible commercialization of the process.

[II] MATERIALS AND METHODS

2.1. Collection of substrate.

Food industrial waste like peel, seeds stones etc produced after the processing of different fruits was collected from the different food industries located in Jalgaon city. For our work papaya waste produced after processing of papaya foods for pulp productions from SHEETAL INDUSTRIES was collected, processed and used as substrate for SSF.

2.2. Microorganism

Microorganism was obtained from National Center for Industrial Microorganism (NCIM), a division at National Chemical Laboratory (NCL), Pune. *Aspergillus Niger* with NCIM No.1054 (amylase producer) is maintained on potato dextrose agar (PDA) slants of pH 5.6 at 4^{0}C.The microorganism is subcultured at regular interval in the department laboratory.

2.3. Preparation of Substrate

Food industry waste (Fruit waste, Specially Papaya Waste) collected from the SHEETAL INDUSTRIES, Jalgaon was spread on the roof and sun dried for 48 hrs. After sun drying the substrate was collected and oven dried at 70^0 C for 24 hrs. Oven dried substrate was grounded to powder in an Electric grinder. The above grinded substrate taken in 250 ml conical flask or petriplates was moistened with salt solution containing gm / 100 ml : yeast extract 0.3, peptone 0.5, NaCl 1.5,Na2HPO4.2H2O 1.1, NaH2PO4 0.61, KCl 0.3 and MgSO4.7H2O 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 ^{0}C for proper cooking of the substrate and to increase its amenability for microorganisms.

2.4. Inoculum Preparation

Aspergillus niger (NCIM No.1054, amylase producer) spores were transferred aseptically to 100 ml conical flask containing 50 ml of sterilized inoculum medium (sterilized at 121^0C for 15 minutes) containing g/100ml: glucose 2, yeast extract 0.3, peptone 0.5, NaCl 1.5,Na2HPO4.2H2O 1.1, NaH2PO4 0.61, KCl 0.3 and MgSO4.7H2O 0.01 in laminar air flow. The flask was then kept in incubator at 37 ^{o}C for 48 hrs. The homogenous spores suspension (10^6 – 10^7 spores / ml) was used as inoculum.

2.5. Solid State fermentation

After sterilizing, the substrate was cooled to room temperature. Substrate of 10 gm in petriplate and 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.

Aspergillus niger (NCIM No.1054, amylase producer) was inoculated on Papaya waste. After inoculation the flask and petriplates were incubated at 37 ^{0} C for 2 days. The SSF media flasks and petriplates were gently shaken after every 12 hrs for uniform mixing of the substrate and microorganism.

2.6. Enzyme Extraction

After incubation, the fermented papaya 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 from the petriplate, was first 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.

2.7. 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.

2.8. Parameters

The effect of varying particle size, extraction pH, incubation pH, incubation temperature, activity temperature, incubation period, moisture content, peptone and yeast extract content on the production of α-amylase was investigated.

[III] RESULTS AND DISCUSSION

3.1. Effect of particle size on Enzyme activity

Effect of particle size on enzyme activity (amylase) was studied by taking papaya waste as substrate. After grinding the substrate the substrate of different particle size from 0.100 mm to 2 mm was taken to study the effect of particle size on enzyme activity. Sieve shaker was used to separate the substrate particle of different size. Sieves of different mesh size arranged in a decreasing order of mesh size as 2 mm , 1.4 mm ,1 mm , 0.850 mm, 0.425 mm, 212mm, 106 mm were mounted on a vibrator. Substrate of different particle size was considered based on under size of particle size. Substrate of each particle size was taken in conical flask (250 ml)

and solid state fermentation was carried out for 48 hrs. at 37 ^{0} C. The crude enzyme was extracted; a reading of each particle size was recorded for enzyme activity. The mean reading of enzyme activity against particle size is shown in Fig No1. Larger particles provide better respiration/aeration efficiency due to increase of interparticle space. In contrast, a small substrate particle may result in substrate accumulation, which may interfere with microbial respiration/aeration and therefore result in poor growth and enzyme production. Decrease in particle size from 2mm to 0.850mm shows increase in enzyme activity, but further decrease in particle up to 0.106mm decreases enzyme activity. Optimal activity of 41.22 IU/min was seen in 0.850mm particle size.

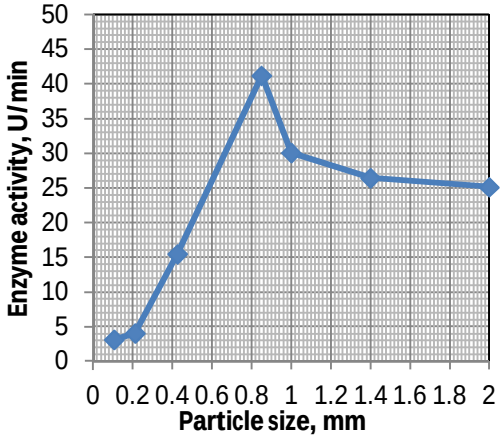

Fig No: 1 Effect of particle size on enzyme activity

Bilan Balkan and Figen Ertan, 2007 produced α-amylase from *Penicillium chrysogenum* from various agriculture by products and got optimum enzyme activity for 1mm particle size [3]. Krishna C. and Chandrasekaran M. 1996 reported production of α-amylase by *Bacillus subtilis* from banana fruit stalk and got optimum activity for 0.4mm particle size [7].

To investigate the effect of extraction pH, incubation pH, incubation temperature, extraction temperature, incubation period, moisture content, peptone and yeast extract content on enzyme activity, 2mm particle size was used for the solid state fermentation.

3.2. Effect of extraction pH on enzyme activity

Solid state fermentation was performed to check the effect of extraction pH of enzyme activity. Crude enzyme was extracted by using buffers of different pH from 4 to 9. Acetate buffer of 0.2M was used for pH 4 and 5, Phosphate buffer of 0.1M was used for pH 6, 7, 8 and Glycine buffer of 0.2M was used for pH 9.The enzyme activity was recorded to study the effect of pH of extracting buffer and also to optimize the condition for pH. Increase in the hydrogen ion concentration considerably influences the enzyme activity. Each enzyme has an optimum pH at which the activity is maximum. Hydrogen ions influences the enzyme activity by altering the ionic charges on the amino acids particularly at the active site, substrate etc. Increase in pH from 4 to 6 increases enzyme activities, further increase in pH up to 9 decreases enzyme activity as shown in Fig No.2. Optimal activity of 13.39 IU/min was observed at pH 6.

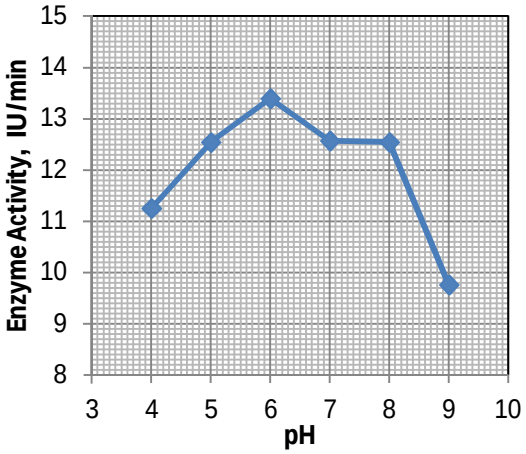

Fig No:2 Effect of extraction pH on enzyme activity

3.3. Effect of initial pH on enzyme activity

Solid state fermentation was performed to study the effect of incubation pH on enzyme activity. Among the physicochemical parameters, pH of the growth medium plays an important role by inducing morphological changes in the organism and in enzyme secretion. Variation of pH results due to substrate consumption (eg: protein hydrolysis) and metabolite production like organic acids. Increase in pH from 4 to 6

increases enzyme activity, further increase in pH up to 9 decreases activity as in Fig No. 3. Optimal activity of 14.61 IU/min was observed at pH 6. Krishna C. and Chandrasekaran M.1996 reported production of α-amylase by *Bacillus subtilis* on banana fruit stalk and got optimum activity at pH 7.0 [7]. Shaista Kokab *et al* 2003 reported production of α-amylase by *Bacillus subtilis* utilizing banana peel and got optimum activity at pH 7.0 [15]. Suganthi R. *et al* 2011 reported production of α-amylase on wheat substrate by *Aspergillus niger* and got optimum activity at pH6.5 [16]. Swetha Sivarama Krishnan *et al* 2007 reported production of α-amylase by *Aspergillus oryzae* on different oil cakes and got optimum activity at pH 5.0 [17]. Mrudula S. *et al* 2011 reported production of α-amylase by thermophilic *Clostridium thermosulforegenes* SVM17 on wheat bran and got optimum activity at pH 7.5 [9]. This indicates that the optimum pH is varying depending on the type of organism used for α-amylase production.

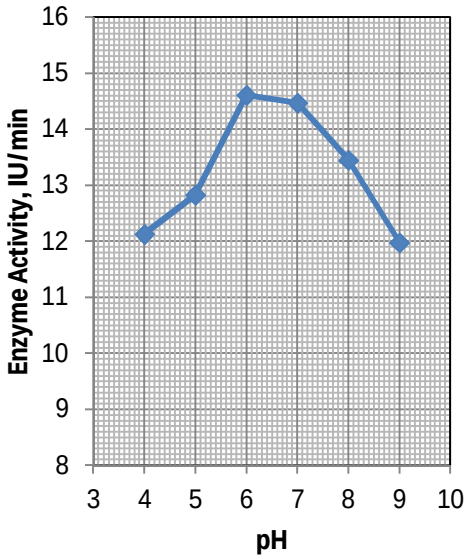

Fig No:3 Effect of initial pH on enzyme activity

3.4. Effect of incubation temperature on enzyme activity

Solid state fermentation was performed to study the effect of incubation temperature on enzyme activity. Incubation temperatures used were 20^0 C, 25^0 C, 30^0C, 35^0C, 40^0C, 45^0C and 50^0C. After inoculation of substrate, it was kept in incubator at different temperature for 2 days. Crude enzyme was extracted and activity was measured as shown in Fig No. 4. Bacterial cells have various mechanisms that allow them strictly to control enzyme excretion. Change in the nature of cell envelope can affect the release of extracellular enzymes to the culture medium. Temperature is one of the factors that induce such changes on cell membrane and cell wall. Increase in temperature from 25^0C to 50^0C, decreases enzyme activity, whereas increase in temperature from 20^0 C to 25^0 C increased the enzyme activity. The optimum enzyme activity obtained was 16.17U/min at 25^0C. The microorganism used was mesophile. Asghar Muhammad *et al* 2000 reported optimum activity of α-amylase by *Arachniotus sp*. using waste bread medium at 32^0 C[1]. Krishna C.and Chandrasekaran M. 1996 reported production of α-amylase by *Bacillus subtilis* on banana fruit stalk and got optimum activity at 35^0C [7]. Shaista Kokab *et al* 2003 reported production of α-amylase by *Bacillus subtilis* utilizing banana peel and got optimum activity at 35^0C. Suganthi R. *et al* 2011 reported production of α-amylase by *Aspergillus niger* and got optimum activity at 30^0C(black gram bran) and 37^0C(ground nut oil cake) [16]. Thus incubation temperature for optimum activity of enzyme also varies with the type of organism.

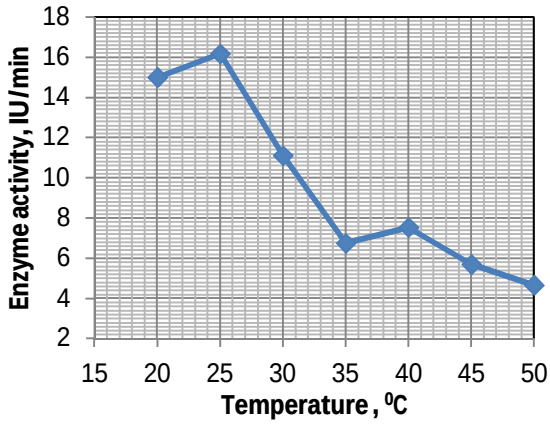

Fig No:4 Effect of incubation temperature on enzyme activity

3.5. Effect of extraction temperature on enzyme activity

Solid state fermentation was performed to study the effect of extraction temperature on enzyme activity. Temperatures used were 25^0 C, 30^0C, 35^0C, 40^0C, 45^0C and 50^0C. After extraction of enzyme, activity was measured at different temperature. Enzyme activity increases with increase in temperature to a maximum and then declines. Increase in temperature results in higher activation energy of the molecules and more molecular collision and interaction of the reaction to proceed faster. When enzymes are exposed to a temperature above maximum denaturation leads to dearrangement in the native structure of the protein and active site, which results in inactivation of enzymes. Increase in temperature from 30^0C to 50^0C decreases enzyme activity, whereas increase in temperature from 25^0C to 30^0C increases enzyme activity as shown in Fig No. 5. The optimum enzyme activity obtained was 16.37IU/min at 30^0C. Enzyme extracted was very sensitive to temperature increase from its optimal value. Drastic decrease in enzyme activity was observed when temperature was increased from 30^0C to 35^0C.

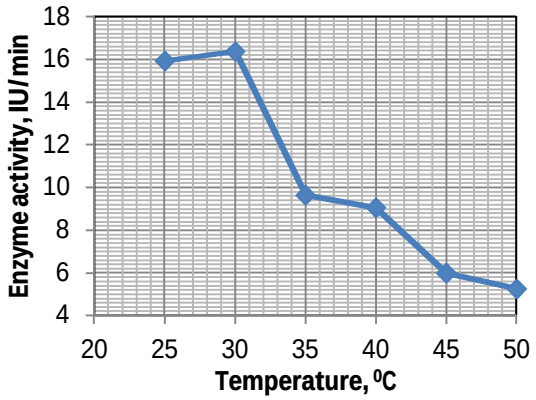

Fig No: 5 Effect of extraction temperature on enzyme activity

3.6. Effect of incubation period on enzyme activity

Solid state fermentation was performed by varying incubation period from 2 to 14 days at 37^0C. Increase in incubation period from 2 days to 6 days increases enzyme activity, whereas increase in incubation period from 6 days to 20 days showed decrease in enzyme activity as shown in Fig No. 6. Optimum activity of 25.73 IU/min was observed at 6^{th} day of incubation. Gradual decrease in enzyme activity was observed during incubation period from 6^{th} day to 12^{th} day due to availability of desired moisture in the substrate, whereas drastic decrease in enzyme activity was observed after 12^{th} day due to decrease in moisture content of the substrate. Asghar Muhammad *et al* 2000 reported optimum activity of α-amylase by *Arachniotus sp.* using waste bread medium at 32^0c for 48hr incubation [1]. Dhanya Gangadharan *et al* 2006 reported optimum incubation period of 72hrs for production of α-amylase by *Bacillus amyloliquefaciens* [4]. Shaista Kokab *et al* 2003 reported optimum activity at 24^{th} hrs of incubation for the production of α-amylase by *Bacillus subtilis* utilizing banana peel [15]. Vasudeo Zambare 2010 reported optimum activity at 120^{th} hrs of incubation for production of α-amylase by *Aspergillus oryzae* on rice husk [19]. Suganthi R. *et al* 2011 reported production of α-amylase by *Aspergillus niger* and got optimum activity at 2^{nd} day (gingely oil cake), 5^{th} day(black gram bran) and 6^{th} day(ground nut oil cake) [16]. Swetha Sivarama Krishnan *et al* 2007 reported production of α-amylase by *Aspergillus oryzae* on different oil cakes and got optimum activity at 72 hrs of incubation [17].

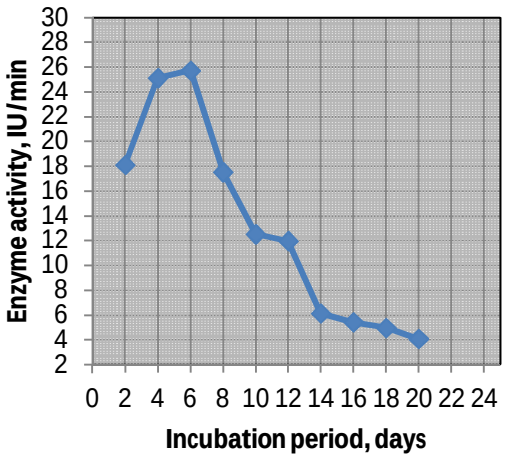

Fig No: 6 Effect of incubation period on enzyme activity

3.7. Effect of initial moisture content on enzyme activity

Grinded substrate of 2mm particle size was taken to study effect of moisture content on enzyme activity. Substrate was cooked with salt solution by adding salt solution (w/v) to get different moisture content (50 to 120%). Initial moisture contents of the substrate are known to critically influence microorganism growth and enzyme production in solid state fermentation. Presence of water in the substrate makes the nutrients more easily accessible for microorganism growth. Moreover, water has an impact on physicochemical properties of the substrate, which in turn affect the enzyme production. Higher water causes reduction in enzyme yield due to steric hindrance of the growth of the producer strain by reducing porosity of the solid matrix, causes particles to stick together thus interfering adversely the oxygen diffusion in the substrate. Lower moisture content causes reduction in solubility of nutrients of the substrate, low degree of swelling and high water tension. Increase in moisture content from 50% to 100% increases enzyme activity, further increase in moisture content of substrate from 100% to 120% decreases enzyme activity as shown in Fig No.7. Optimum enzyme activity of 25.14 IU/min was observed at 100% moisture content of the substrate. Dhanya Gangadharan *et al* 2006 reported optimum enzyme activity at 85% initial moisture content for production of α-amylase by *Bacillus amyloliquefaciens* [4]. Krishna C. and Chandrasekaran M. 1996 reported production of α-amylase by *Bacillus subtilis* on banana fruit stalk and got optimum activity at 70% moisture content [7]. Vasudeo Zambare 2010 reported optimum activity at 100% moisture content for production of α-amylase by *Aspergillus oryzae* on rice husk [19]. Swetha Sivarama Krishnan *et al* 2007 reported production of α-amylase by *Aspergillus oryzae* on different oil cakes and got optimum activity at 60% moisture content [17].

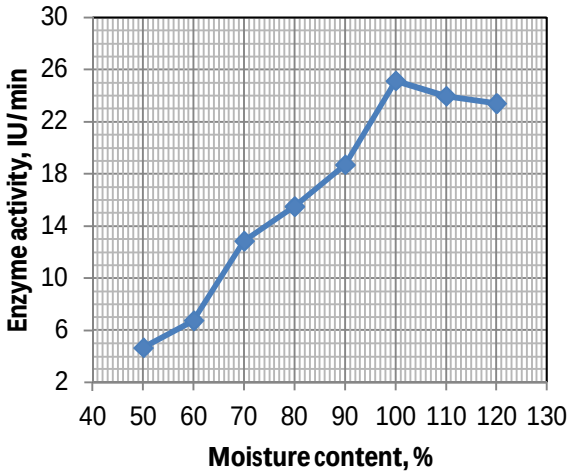

Fig No: 7 Effect of initial moisture content on enzyme activity.

3.8. Effect of peptone and yeast extract content on enzyme activity

Productions of hydrolytic enzymes are enhanced by the additional nitrogen source like peptone and yeast extract. In this work effect of different concentrations of peptone and yeast extract was checked on enzyme activity. Increase in peptone content from 0.1 gm to 0.6 gm and yeast extract content from 0.06 to 0.42 gm increases enzyme activity, further increase in peptone content from 0.6 to 1.0gm and yeast extract to 0.48gm decreases enzyme activity as shown in Fig No.8 and Fig No.9. Optimum activity of 30.42IU/min was observed at 0.6gm of peptone content and 32.45IU/min at 0.42gm of yeast extract. H.Anto

et al 2006 reported optimum activity at 0.02gm of peptone content on agro industrial waste by *Aspergillus Sp* [5]. Krishna C. and Chandrasekaran M. 1996 reported production of α-amylase by *Bacillus subtilis* on banana fruit stalk and got optimum activity at 0.5gm peptone content [7]. Ramachandran et al 2004 reported optimal activity at 0.05gm peptone content using *Aspergillus oryzae* on different oil cakes [12]. Vasudeo Zambare 2010 reported optimum activity at 0.1gm of peptone and yeast extract content for production of α-amylase by *Aspergillus oryzae* on rice husk [19]. Haq NawazBhatti *et al* 2007 reported optimum activity at 0.1gm of peptone and yeast extract content for production of glucoamylase by *Fusarium solani* on wheat bran [6].

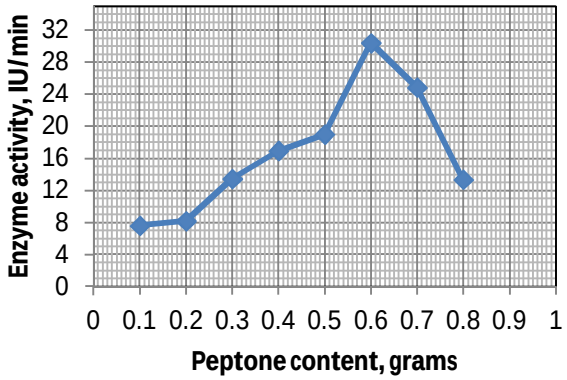

Fig No: 8 Effect of peptone content on enzyme activity

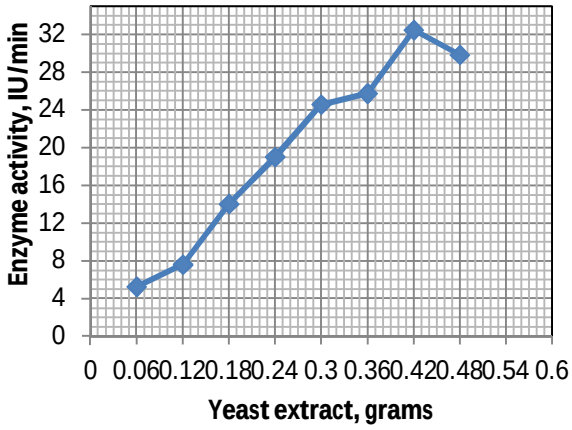

Fig No: 9 Effect of yeast extract content on enzyme activity

[IV] CONCLUSION

SSF offers numerous advantages over submerged fermentation; these include high productivity, relatively higher concentration of products, less effluent generation and simple fermentation equipment. Food industry waste (papaya waste) provides a low cost feed stock for biological production of amylase. In present study, *Aspergillus niger* (NCIM No.1054, amylase producer) was obtained from NCIM, NCL, Pune. The optimum activity of enzyme 41.22IU/min was obtained for 0.850 mm particle size, 37^0 C incubation temperature and 48 hr incubation period. Effect of extraction pH and incubation pH on enzyme activity was studied and it is found that optimum extraction pH is 6 with 13.39IU/min enzyme activity and incubation pH 6 with 14.61IU/min enzyme activity respectively. Effect of incubation and activity temperature was considered to study effect on enzyme activity. For amylase production from papaya waste, the optimum temperature was 25^0C with 16.17IU/min and 30^0 C with 16.37IU/min of enzyme activity respectively. Optimal activity of 25.73IU/min was observed for incubation period on 6^{th} day. For moisture content of 100% optimum activity was 25.14IU/min. For peptone and yeast extract content, optimum activity was 30.40IU/min for 0.6 gm of peptone content and 32.45IU/min for 0.42 gm of yeast extract content. Thus papaya waste could be a potential, economic source for the production of α-amylase by solid state fermentation.

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