International Journal of
Scientific Engineering and Technology Research

ISSN 2319-8885
Vol.03,Issue.04,
April-2014,
Pages:0564-0573

www.semargroup.org,
www.ijsetr.com

Production Optimization of Alpha Amylase from Bacillus Altitudinis

YASHAB KUMAR[1], PRADEEP KUMAR SINGH[2], AJAY KUMAR SINGH[3], HARISON MASIH[4], JYOTSNA KIRAN PETER[5], JANE C. BENJAMIN[6], SANAT RATH[7]

[1]Research Scholar, Dept of Microbiology and Fermentation Technology, Sam Higgibottom Institute of Agriculture, Technology & Sciences, Allahabad, UP, India.
[2]Research Scholar, Dept of Microbiology and Fermentation Technology, Sam Higgibottom Institute of Agriculture, Technology & Sciences, Allahabad, UP, India.
[3]Research Scholar, Dept of Microbiology and Fermentation Technology, Sam Higgibottom Institute of Agriculture, Technology & Sciences, Allahabad, UP, India, Email: singhs_ajay@rediffmail.com.
[4]Research Scholar, Dept of Microbiology and Fermentation Technology, Sam Higgibottom Institute of Agriculture, Technology & Sciences, Allahabad, UP, India.
[5]Research Scholar, Dept of Microbiology and Fermentation Technology, Sam Higgibottom Institute of Agriculture, Technology & Sciences, Allahabad, UP, India.
[6]Research Scholar, Dept of Microbiology and Fermentation Technology, Sam Higgibottom Institute of Agriculture, Technology & Sciences, Allahabad, UP, India.
[7]Research Scholar, Dept of Microbiology and Fermentation Technology, Sam Higgibottom Institute of Agriculture, Technology & Sciences, Allahabad, UP, India.

Abstract: α-Amylases are a class of starch degrading enzymes catalyzing the hydrolysis of internal α-1,4-O-glycosidic bonds in polysaccharides and plays a pivotal role in a variety of areas like use as digestives, for the production of ethanol and high fructose corn syrup, detergents, desiring of textiles,modified starches, hydrolysis of oil-field drilling fluids, and paper recycling. In the present work, submerged fermentation (SmF) for *α*-amylase production has been used. Bacillus ultitudinis has been used for the production of alpha amylase and was tested using submerged fermentation. Bacillus altitudinis showed maximum enzyme production at 40°C for 48 hours at pH 7.0. The Peak enzyme production was obtained while supplementing starch as carbon source, ammonium sulphate and Yeast extract and 2.5% salt concentration.

Keywords: α Amylase, Bacillus Altitudinis, Submerged Fermentation.

I. INTRODUCTION

Nowadays, the application of enzymes in industrial sector is increasing due to increase of industries especially in food, beverages, and textile, leather and paper industries. Amylases constitute a class of industrial enzymes having approximately 25% of the enzyme market (Sidhu et al., 1997; Rao et al., 1998). Amylases are characterized by their ability to hydrolyze glycoside linkages in polysaccharide. Alpha amylase [α-amylase, endo1, 4-α-D-glucan glucohydrolase, EC 3.2.1.1] is an extracellular enzyme acts on starch and degrade it into disaccharide and trisaccharide (Crabb and Mitchinson, 1997). Alpha amylase hydrolyses the internal α-1, 4 linkages in starch in a random fashion leading to the formation of soluble maltodextrins, maltose, and glucose. This enzyme is extensively used in starch liquefaction, brewing, food, paper, textile and pharmaceutical industries (Akpan et al.,2004; Thippeswamy et al., 2006; Gangadharan et al., 2008; Rajagopalan and Krishnan, 2008; Rasiah & Rehm, 2009). Amylases have potential application in a number of industrial processes such as in the food, textiles, paper industries, bread making, glucose and fructose syrups, detergents, fuel ethanol from starches, fruit juices, alcoholic beverages, sweeteners, digestive aid and spot remover in dry cleaning (Kathleen et al., 1996).

Alpha amylase can be derived from various sources such as plants, animals and microorganisms. The major advantages of using microorganisms for production of amylases are in economical bulk production capacity and microbes are also easy to manipulate to obtain enzyme of desired characteristics (Vidyalakshmi et al., 2009). Several species of Bacillus produces a wide range of extra cellular enzymes of which amylases are of significant industrial importance (Pandey et al., 2000). Thermophilic microorganisms have gained a great deal of attention recently (Becker et al., 1997; Beg et al., 2000). Enzymes from these microorganisms are of special interest since they are not usually denatured by high temperatures and are even active at elevated temperatures (Adams and Kelly, 1998;

Fitter and Heberle, 2000). They are usually stearo thermophilus**,** Bacillus subtilis, Bacillus licheniformis, Bacillus amyloliquefaciens(Boyer and Ingle, 1972; Sajedi et al., 2005). Bacillus species produce a large variety of extra cellular enzymes, such as amylases, which have significant industrial importance (Cordeiro et al., 2003). Members of the genus Bacillus are heterogeneous and they are very versatile in their adaptability to the environment. There are various factors that influence the nature of their metabolic processes and enzymes produced. A great deal of attention is being given to thermophilic and extremely thermophilic microorganisms and their enzymes (Ajayi and Fagade, 2006; Oyeleke and Oduwole, 2009).

Production of alpha amylase is greatly affected by the cultivation method. Alpha amylase can be produced both by solid state (Omori et al., 1994; Akpan and Adelaja, 2004) and submerged fermentation technique (Castro et al., 1992; Haq et al., 1997; Allan et al., 1997; Kim et al., 1997; Vanova et al., 2001). The submerged culture method of producing amylases would have definite advantages when the product could be employed directly without concentration or purification (Julian et al., 1995). Submerged fermentation has been used for the production of industrially important enzymes because of the case of handling and greater control of environmental factors such as temperature and pH. Due to the advantages offered by the application of thermostable hydrolases such as reduction of reaction time and contamination risk which provide considerable energy saving, there is always a requirement for hydrolases capable of functioning at elevated temperature. Furthermore in order to meet this demand the identification of new strain and development of low cost fermentation process are necessary. Therefore in view of the above present study reports the production and optimization of alpha amylase from novel Bacillus ultitudinis (MCCB ..) isolated from soil.

II. MATERIAL AND METHODS

A. Place of work

The study was carried out in the Department of Microbiology and Fermentation Technology, Sam Higginbottom Institute of Agriculture, Technology and Sciences, (Deemed-to-be-University), Allahabad , (Uttar Pradesh), India.

B. Procurement of microorganism and its maintenance

The bacterial strain Bacillus altitudinis (FN667875) was earlier isolated from the soil and then 16S rDNA was performed and sequence was submitted in NCBI sequence submission data bank The Bacterial culture was obtained from Microbial Culture Collection Bank (MCCB), Department of Microbiology and Fermentation Technology. The culture was maintained in Nutrient Agar Medium and subcultured time to time.

C. Inoculum development

The Inoculum was prepared by inoculating the bacterial culture into the growth medium (nutrient broth) for 16 hours and then 2 ml of liquid culture was added to the production medium.

D. Production of Alpha Amylase Under Submerged Fermentation

The amylase production was carried out using the basal media comprised in g/L; starch 10, peptone 10, Yeast extract 20, manganese chloride 0.015, calcium chloride 0.05 Potassium di hydrogen phosphate 0.05, magnesium sulphate 0.25, Ferrous sulphate 0.01 and pH was maintained 7.0±0.2 The production medium was sterilized by autoclaving at 121°C for 15 minutes. The production media was inoculated with 2 ml inoculum of Bacillus ultitudinis, aseptically and incubated at 40±2°C for 48 hour. After incubation the fermented broth was subjected to centrifugation at 7000 rpm for 20 minute at 4°C. The cell free, clear supernatant was used as crude enzyme preparation and further assayed for enzyme activity.

1. Enzyme assay by DNS method

The crude enzyme obtained after centrifugation was assayed for amylase activity by measuring the release of reducing sugar following the DNS method (Fisher and Stein, 1961).

2. Preparation of Maltose standard curve

A stock solution of 1mg/ml maltose was prepared in 0.1M sodium phosphate buffer (pH 7.0) and diluted. The graph was plotted between different concentration of maltose and their respective O.Ds as given below (Fig 1)

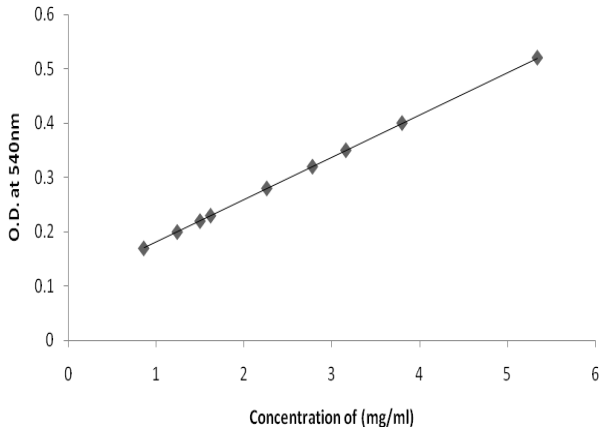

Figure 1: Maltose standard curve.

3. Enzymatic assay of alpha amylase

One ml of crude enzyme supernatant was taken in test tube and 1.0 ml of substrate (starch solution) was added in test tube. The test tubes were covered and incubate at 35°C for 15 minutes in water broth. Then 2.0 ml of DNS reagent

was added in each tube and the reaction was stopped by boiling the reaction mixture in water bath for 10 minutes. After cooling at room temperature, the absorbance (O.D) was measured at 540 nm by spectrophotometer and the released sugar was determined from maltose standard curve. One unit of amylase activity was defined as the amount of enzyme that released 1µmol reducing sugar equivalent maltose per minute under the assay condition.

E. Optimization of process parameters for alpha amylase production using one variable at a time (OVAT)

The optimization of alpha amylase production was evaluated by varying incubation temperature, pH, fermentation time, carbon sources, nitrogen sources and NaCl concentrations.

1. Effect of incubation time on alpha amylase production

The optimum incubation time for alpha amylase production was determined by inoculating 50 ml production media with the bacterial inoculum and incubated at 40±2°C. The samples were withdrawn at 24 hrs time intervals i.e. 24, 48, 72, 96, 120 and 144 hours. The enzyme activity was determined for each time by the standard assay method.

2. Effect of temperature on alpha amylase production

The optimum temperature for alpha amylase production was determined by inoculating 50 ml broth media with the bacterial inoculum and incubated at different temperatures (30, 40, 50, 60, 70, 80, 90 and 100°C for 48 hours. The enzyme activities were determined at respective temperature by the standard assay method.

3. Effect of pH on alpha amylase production

To determine optimum pH for maximum alpha amylase production, 50 ml broth media was adjusted with buffers at different pH (5, 6, 7, 8, 9, 10 and 11) and inoculated with the inoculum and incubated at 40°C±2°C for 48 hour. After incubation, the enzyme activity at each pH was assayed by standard assay procedure.

F. Optimization of Nutrient parameters for alpha amylase production

1. Effect of different carbon sources on α amylase production

Each 50 ml production media supplemented with the additional carbon sources such as starch, sucrose, fructose, dextrose, maltose and lactose at 1% (w/v) level was inoculated with the inoculum of Bacillus ultitudinis and incubated at 40±2°C for 48 hour and pH 7.0. The standard assay was performed to determine maximum alpha amylase production with varying the carbon sources.

2. Effect of different nitrogen sources on α amylase production

Each 50 ml broth media supplemented with the additional inorganic nitrogen sources such as ammonium chloride, sodium nitrate, ammonium sulphate, ammonium nitrate and organic nitrogen sources such as beef extract and yeast extract were inoculated with the test organism incubated at 40±2°C for 48 hours at pH 7.0. The standard assay was performed to determine maximum alpha amylase production with varying inorganic and organic nitrogen sources.

3. Effect of different NaCl concentrations α amylase production

Production media (50 ml) was supplemented with the additional concentrations of NaCl concentrations and inoculated with the test organism and incubated at 40±2°C for 48 hours at pH 7.0. Maximum alpha amylase production with varying salt concentrations was determined by standard method of enzymatic assay.

III. STATISTICAL ANALYSIS

The optimization of growth parameters such as incubation time, temperature, pH, and nutritional parameters such as carbon sources, nitrogen sources and salt concentration for alpha amylase production was analyzed using ANOVA (Analysis of variance technique) one way classification and conclusion was drawn on the basis of analysis of variance technique (Fischer and Yates, 1968) at 5% level of significance.

IV. RESULTS AND DISCUSSION

A. Optimization of Production Parameters for bacterial alpha amylase production

1. Effect of incubation time on α-amylase production

The enzyme production by the bacterial strain Bacillus altitudinis was studied on the basis of incubation time in submerged fermentation. The significant alpha amylase yield was obtained for the incubation period from 48-120hrs. It was observed that the enzyme production from the bacterium was found maximum at 48 hour. An increase in the enzyme production was observed from 24h to 48h. After 48h of incubation a decreasing trend of enzyme

TABLE 1: EFFECT OF INCUBATION TIME ON A-AMYLASE PRODUCTION BY BACILLUS ALTITUDINIS

Incubation Time (h)	Enzyme activity (Uml^{-1})
24	2.05
48	4.26
72	3.96
96	3.53
120	2.73
144	2.43

$F_{cal} = 33.18 > F_{tab}$ (5%) = 3.11 (due to time)

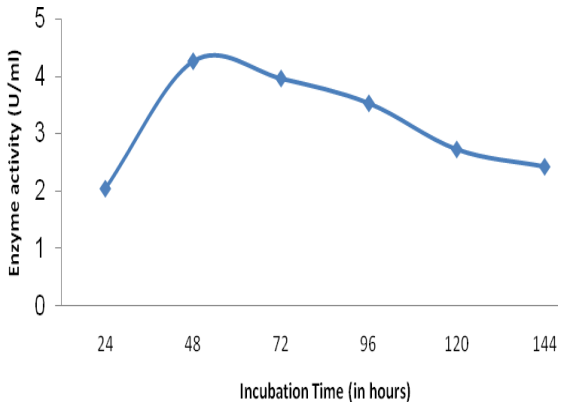

Fig 2: Amylase activity at different incubation time

activity was observed (Table 1 and Figure 2). The effect of incubation time on alpha amylase production was found to be statistically significant. (F_{cal} 33.18 > F_{tab} 3.11 Due to incubation time) .

The results are similar to the study conducted by Jogezai et al. (2011) on α-amylase production by Bacillus subtilis in shake flask for different intervals of time (0 to 72 h). The production of enzyme was reached maximum at 48 h after inoculation. Further increase in incubation period however, did not show any significant increase in enzyme production rather it was decreased. This is because the cells would have reached decline phase with lowered enzyme synthesis. Further Lealem and Gashe (1994) also reported decreased production of the enzyme with increase in incubation period. It might be due to the depletion of the nutrients, death phase of organism or due to the production of amylase in the medium as suggested by. This result was also supported by Oyeleke et al. (2010) where the optimum incubation period on the yield of amylase enzyme was found at 48 h. After this a decline in amylase activity was obtained. This result was also in agreement with Kumar and Sivasudha (2011) for the amylase production by solid state technology. The similar findings were obtained by Haq et al. (2010) for production of α-amylase from Bacillus amyloliquefaciens. The result revealed that after 48 h incubation decreased in enzyme yield might be due to the denaturation of enzyme caused by interaction with other components in the medium.

2. Effect of temperature on α-amylase production

The enzyme activity for the bacterial strain Bacillus altitudinis was studied on the basis of parameter i.e. temperature in submerged fermentation. In this present work the maximum amylase activity was studied for the temperature from 30°C-100°C. It was observed in the present study that the maximum enzyme production from the bacterium was found to be maximal after 48 hour of time at 40°C (Table 2 and Figure 3). The effect of temperature on alpha amylase production was found to be statistically significant (F_{cal} 30.31 > F_{tab} 2.66 Due to temperature) at 5% level of significance. Bhaskara et al. (2011) found optimum production of amylase by Bacillus marini at 40°C and as the temperature increased or decreased, there was gradual decrease in the enzyme activity. At 50°C, the production of amylase was extremely low. It might be due to inhibition of bacterial growth at high temperature and hence, enzyme formation was also prohibited. Similar finding was also reported by Riaz et al. (2003) when study was carried out on production of α-amylase by Bacillus subtilis GCUCM-25 at 30, 35, 40, 45, 50, 55 or 60°C in rotary incubator shaker.

TABLE 2: EFFECT OF TEMPERATURE ON A-AMYLASE PRODUCTION BY BACILLUS ALTITUDINIS

Temperature(°C)	Enzyme activity(U/ml)
30	2.97
40	5.64
50	4.65
60	4.14
70	3.66
80	3.11
90	2.64
100	2.22

F_{cal} = 30.31 > F_{tab} (5%) = 2.66 (due to temperature)

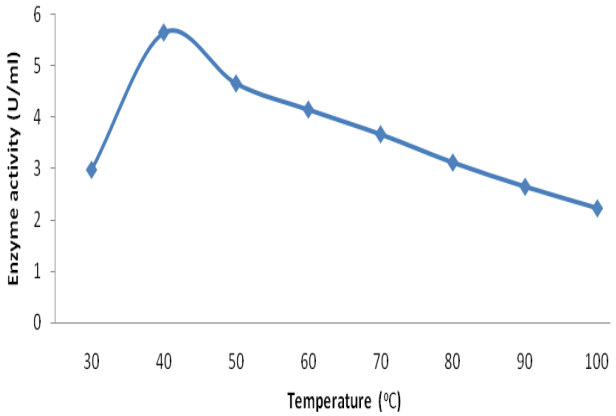

Figure 3: Amylase activity at different temperature

The maximum production of α-amylase was obtained at 40°C. The production of the enzyme decreased with increased temperature. The production of the enzyme was greatly inhibited at lower temperature (30°C). The effect of temperature on activity of amylase produced by Bacillus megaterium was found to be maximum at 40°C followed by a sharp decrease in amylase activity at 50°C reported by Oyeleke et al. (2010) which is similar to the present study. (Jogezai et al., 2011) also reported the maximum production of α-amylase at 40°C by using Bacillus subtilis. As the incubation temperature was increased, the production of the enzyme was decreased. According to Liu and Xu (2008)

study the strain Bacillus aquimaris VITP4 exhibited maximum enzyme production at 40°C. Although growth was observed in the temperature range 30°C to 60°C, it was found maximum at 40°C indicating one-to-one correlation between enzyme production and biomass, which clarifies that the enzyme production is growth dependent. These results were in agreement with the present reports for Bacillus ultitudinis**.** From these results, the enzyme seemed to have considerable thermostability, which can be favourable in industrial operations for traditional brewing and food processing.

3. Effect of pH on α-amylase production

The pH of the production medium plays an important role in microbial growth and hence influences the enzyme production. In the present study the enzyme activity for different pH ranging from 5.0-11.0 was determined by keeping the optimum time and temperature combination. The maximum amylase production was found at pH 7.0. After pH 7 a gradual decreasing trend of enzyme yield was observed. Since there was no constant change in the amylase activity with the increase or decrease of pH, the data was found to be statistically significant. (F_{cal} 21.02 >F_{tab} 2.85 at 5% level of significance)(Table 3 and Figure 4)Bhaskara et al. (2011) reported that the pH of media is one of the regulatory parameters during fermentation. Highest amylase enzyme production was observed highest at pH 7.0. A gradual decrease in the enzyme yield was obtained towards pH below 5.0 and above 10. Similar pH optimum for amylase production was reported by (Oyeleke et al., 2010, Shafaat et al., 2011 and Vaseekaran et al., 2011).

TABLE 3: EFFECT OF PH ON A-AMYLASE PRODUCTION BY BACILLUS ALTITUDINIS

pH	Enzyme activity (Uml^{-1})
5	2.14
6	3.07
7	**5.00**
8	4.38
9	3.53
10	2.73
11	2.43

F_{cal} = 21.02 > F_{tab} (5%) = 2.85 (due to pH)

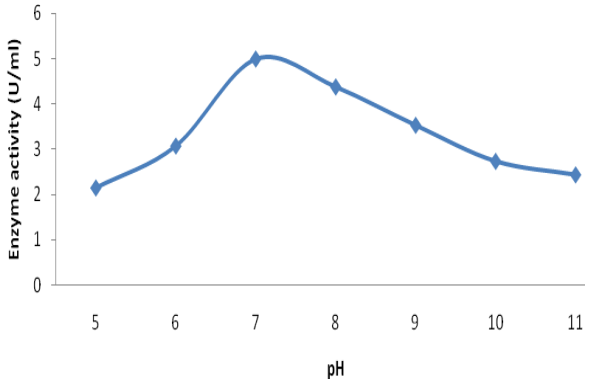

Figure 4: Amylase activity at different pH on α-amylase production

The optimum activity of the enzyme at low pH values (pH 6.5–7.0) is very important from the viewpoints of industrial application. The use of liquefying amylases that are active and stable around the saccharification pH is attractive because it could avoid or reduce the use of acid to lower the pH from liquefying to saccharifying range, and also simplify the procedures during downstream processing. Further, the use of α-amylases that operate at lower pH values reduce the formation of some by-products, such as maltulose, which is usually produced at higher operation pH (Goyal et al., 2005). However, different organisms have different pH optima and decrease or increase in pH on either side of the optimum value results in poor microbial growth (Ramesh and Lonsane, 1991). In contrast, maximum activities at pH range of 4.8 and 9.2 were reported for the production of amylase from B. licheniformis from a cassava processing waste (Oguntimehin, 1998). Ellaiah et al. (2002) showed that at higher pH, the metabolic activity of the bacterium would be suppressed thus inhibiting enzyme production. (Carvalho et al., 2008) carried out enzyme production at neutral pH and reported that a slight raise in the pH probably due to the release of various metabolites as the growth proceeds that favours the production of enzyme. Variation in pH resulted due to substrate consumption (e.g. protein hydrolysis) and/or metabolite production (e.g. organic acids). They are indicators of changes in metabolic activity (Bellon et al., 2003). (Kunamneni et al., 2005) also reported that the pH change observed during the growth of microbes also affects product stability in the fermentation medium.

4. Effect of different carbon sources on α-amylase production

Various carbon sources such as Starch, Sucrose, Fructose, Dextrose, Mannitol and Lactose (1%) were used to supplement the production media. The maximum amylase production was found when production medium was supplemented with starch followed by maltose (Table 4 and Figure 5). Since the data with respect to carbon sources are statistically significant, it can be concluded that variation in different carbon sources would significantly affect the α-amylase production. (F_{cal} 130.96. > F_{tab} 3.11, at 5% level of significance). The addition of carbon source in the form of

either monosaccharide or polysaccharides may influence the production of amylase enzyme. In this present study, the influence of starch was found best carbon source than the other carbon sources. Similar finding was observed by Bhaskara et al. (2011) when amylase production was optimized using different sugars at 1% (w/v) concentration. Bacillus marini showed the maximum enzyme activity in the presence of starch as carbon source, whereas, the minimum enzyme activity was observed in the presence of dextrose.

TABLE 4: EFFECT OF DIFFERENT CARBON SOURCES ON ALPHA AMYLASE PRODUCTION BY BACILLUS ALTITUDINIS

Carbon sources (1%)	Enzyme activity (Uml^{-1})
Fructose	1.76
Sucrose	2.45
Lactose	2.37
Starch	**5.55**
Maltose	4.20
Dextrose	2.53

$F_{cal} = 130.96 > F_{tab}$ (5%) = 3.11 (Due to carbon source)

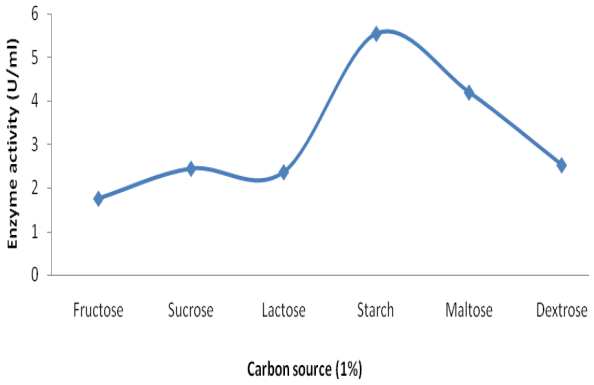

Figure 5: Amylase activity at different carbon sources.

Srivastava and Baruah (1986) reported starch as the best substrate based on several aspects such as specific cell growth rate, enzyme activity level, specific enzyme activity level, specific enzyme formation rate, etc. Soluble starch was found as the best substrate for the production of α-amylase by Bacillus stearothermophilus, so starch was selected as substrate for alpha amylase production in submerged fermentation studies. However, the effect of carbon sources changes with the production strain and other conditions. The decrease in the production of enzyme with other carbon sources may be due to catabolite repression. The finding in the present study was in agreement with (Kumar and Sivasudha, 2011) where starch was observed as the best carbon source utilized by the organism. The similar result was also found by Goyal et al. (2005) that the soluble starch as the best carbon source supplement for amylase production by Bacillus lichenifomis and Bacillus sp.I-3**.** Varalakshmi et al. (2008) also observed maximum amylase yield with soluble starch.

5. Effect of nitrogen sources on α-amylase production

Different organic (yeast extract and beef extract) and inorganic nitrogen (ammonium chloride, sodium nitrate, ammonium nitrate and ammonium sulphate) sources were supplemented in the production media to determine the maximum enzyme yield. The maximum α-amylase production was observed with yeast extract (organic) and ammonium sulphate (inorganic) (Table 5 and Figure 6). All other production parameters viz. temperature, incubation time, pH and carbon source were kept constant. Since the data with respect to nitrogen sources are statistically

TABLE 5: EFFECT OF NITROGEN SOURCES ON AMYLASE PRODUCTION BY BACILLUS ALTITUDINIS

Nitrogen source	Enzyme activity ($U\ ml^{-1}$)
Inorganic	
Ammonium chloride	2.10
Sodium nitrate	3.79
Ammonium nitrate	4.10
Ammonium sulfate	4.42
Organic	
Yeast extract	**4.46**
Beef extract	3.54

$F_{cal} = 87.98 > F_{tab}$ (5%) = 3.11 (Due to nitrogen source)

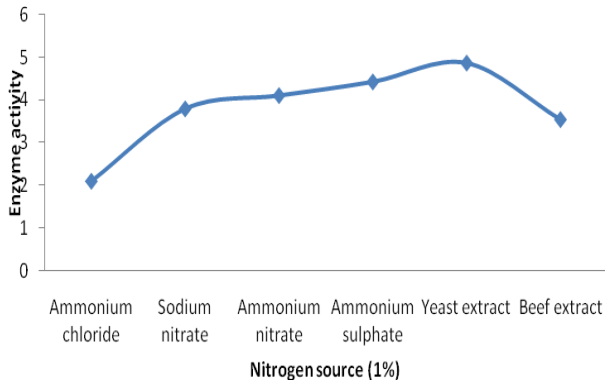

Figure 6: Pattern of enzyme yield with respect to different inorganic and organic nitrogen sources.

significant, it is clear that variation in different organic and inorganic nitrogen sources would significantly affect the amylase production. (F_{cal} 87.98 > F_{tab} 3.11 at 5% level of significance) Nitrogen compounds are secondary energy sources for organisms and play an important role in growth and production of secondary metabolites. The nature of the compound and the concentration used may regulate the production of enzymes. In the present study the supplementation of nitrogen sources on amylase production showed that yeast extract was found to be a better nitrogen source for the production of alpha amylase. Yeast extract was the best nitrogen source for amylase production, probably due to its high content in minerals, vitamins, coenzymes and nitrogen components (Guerra and Pastrana, 2002).

The findings in the present study were in agreement with the studies conducted by Roohi et al, (2011) with yeast extract as best organic nitrogen sources. Teodoro and Martins (2000) also reported similar result when yeast extract was used as nitrogen source in combination with peptone was reported. The results were also in agreement with reports given by Dettori et al. (1992); Narayana and Vijyalakshmi (2008) when yeast extract was used as nitrogen source for Bacillus stearothermophilus and S. albidoflavus respectively. The fact that the maximum growth of Bacillus altitudinis occurs at the highest nitrogen content peptone that exemplified the role of easily fermentable carbon compounds in the medium. The production of primary metabolite by microorganism was highly influenced by their growth, which was determined by the availability of nutrients in the medium. Therefore the improvement of the nutritional value in the medium by the supplementation of organic and inorganic sources will also improve the growth of the bacterial culture and subsequently in the enzyme production. Similarly, enzyme production was more efficient in medium containing organic nitrogen sources, especially yeast extract as compared with inorganic nitrogen sources (Bhattacharya et al., 2011). Earlier studies indicated that organic nitrogen sources were preferred for the production of α- amylase and maximum α-amylase production was observed by yeast extract; peptone or beef extract (Krishnan and Chandra 1982). Santos and Martin (2003) also reported the optimum production of amylase by Bacillus sp. when yeast extract was used as nitrogen sources.

6. Effect of NaCl concentration on α-amylase production

Various concentrations of NaCl such as 0.5%, 1.5%, 2.5% and 3.5% were used to supplement the production media. Among the various concentrations the maximum amylase production was induced when the media was supplemented with 2.5% (Table 6 and Figure 7). Since the data with respect to NaCl concentrations are statistically significant, it can be concluded that variation in different metal ions would significantly affect the amylase production. (F_{cal} 47.76 > F_{tab} 3.11, at 5% level of significance). Sodium chloride is an important nutrient factor for growth and physiological activities. Vijayabaskar et al. (2012) reported 3% NaCl concentration was suitable for the amylase production. Bhaskara et al. (2011) reported enzyme production at different concentrations of NaCl and found optimum enzyme yield at 4.5% NaCl concentrations. Further there was gradual decrease in enzyme production as the NaCl concentration was increased or decreased. Kokab et al, (2003) produced alpha amylase from Bacillus subtilis in solid state fermentation having medium containing 2.0% concentration of NaCl. Broad range of salt tolerable concentrations was reported in halophilic amylases from M. halobia (Onishi and Sonada, 1979) moderate halophile Halomonas meridian (Coronado, et al., 2000), Bacillus dipsosauri (Deutch, 2002) and Halobacterium halobium (Good and Hartman, 1970). A thermophilic Bacillus sp strain SMIA-2 retained 63.4% enzyme yield in 2.0% NaCl (Carvalho, et al., 2008).

TABLE 6: EFFECT OF NACL CONCENTRATIONS ON A-AMYLASE PRODUCTION BY BACILLUS ALTITUDINIS

NaCl Concentrations (%)	Enzyme activity (Uml^{-1})
0.5	1.85
1.5	2.62
2.5	4.00
3.5	3.21

F_{cal} = 47.76 > F_{tab} (5%) = 3.11

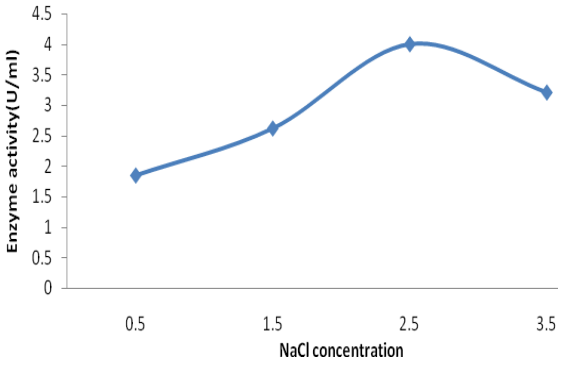

Figure 7: Effect of different NaCl concentration on enzyme yield.

V. SUMMARY AND CONCLUSION

The optimum yield of α-amylase was found at 40°C and 48h and pH 7.0 under submerged fermentation. The data was found to be statistically significant. Among the various

carbon sources, starch supported highest α-amylase production followed by Maltose under submerged fermentation. Ammonium sulphate supported maximal α-amylase production from different nitrogen sources (Organic and inorganic). A NaCl (2.5%) concentration was observed for highest α-amylase production under submerged fermentation. α-amylase produced by Bacillus altitudinis was found to be potent since it could be active at a wide range of pH, Temperature, Carbon, Nitrogen (inorganic and organic) sources and sodium chloride concentrations under submerged fermentation. Therefore the selected strain of Bacillus altitudinis could be beneficial for Industrial purposes after optimizing at large scale production.

VI. ACKNOWLEDGEMENT

The authors thank to the Hon'ble Vice Chancellor, Sam Higginbottom Institute of Agriculture Technology & Sciences (Deemed to be University), Allahabad for granting research work facility and Prof. (Dr.) Rubina Lawrence, Head, Department of Microbiology and Fermentation Technology, for coordinating and giving time to time technical suggestions during the research work.

VII. REFERENCES

[1] Adams, M. W. W. and Kelly, R. M., 1998, Finding and using thermophilic enzymes. Trends Biotechnology, 16: 329-332.

[2] Ajayi, A. O. and Fagade, O. E., 2006, Growth pattern and structural nature of amylases produced by some Bacillus species in starchy substrates. African Journal of Biotechnology, 5(5): 440-444.

[3] Akpan, T., Kawak, Y. S. and Kudo, T., 2004, Production and stabilization of alpha amylase preparation from rice bran solid medium. World Journal Microbiology Biotechnology, 20:47-50.

[4] Akpan, I. and Adelaja, F. A., 2004, Production and stabilization of alpha amylase preparation from ricebran solid medium. World Journal of Microbial Biotechnology, 20(1): 47-50.

[5] Allan, S., Torbbenvedal, B. and Henrik, B. F., 1997, Recombinant alpha amylase mutants and their use in textile desizing, starch liquefaction and washing. International Applied, 12 (5): 205-210.

[6] Becker, P., Abureesh, I., and Markossian, S., 1997, Determination of the kinetic parameters during continuous cultivation of the lipase producing thermopile Bacillus sp. IHI-91 on olive oil. Applied Microbiology Biotechnology, 48: 184-190.

[7] Beg, Q. K., Bhushan, B., Kapoor, M. and Hoondal, G. S., 2000, Production and characterization of thermostable xylanase and pectinase from Streptomyces sp. QG-11-3. Journal of Indian Microbiology Biotechnology, 24: 396-402.

[8] Bellon, M. V., Orliac, O. and Christen, P., 2003, Sensors and measurements in solid-state fermentation. Process Biochemistry, 38: 881–896.

[9] Bhaskara R. K. V., Ashwini, K., Gaurav, K. and Karthik, L., 2011, Optimization, production and partial purification of extracellular α-amylase from Bacillus sp. marini. Archives of Applied Science Research, 3 (1): 33-42.

[10] Bhattacharya, S., Bhardwaj, S., Das, A. and Anand, S., 2011, Utilization of Sugarcane Bagasse for Solid- State Fermentation and Characterization of α- Amylase from Aspergillus flavus. Australian Journal of Basic and Applied Sciences, 5(12): 1012-1022.

[11] Boyer, E. W. and Ingle, M. B., 1972, Extracellular alkaline amylase from a Bacillus species. Journal of Bacteriology, 110: 992-1000.

[12] Castro, G. R., Ferrero, M. A., Abate, A. M., Medex, S. B. and Sineriz, F., 1992, Simultaneous production of alpha and beta amylases by Bacillus subtilis MIR-5 in batch and continuous culture. Literature of Biotechnology, 14: 49-57.

[13] Carvalho, R. V., Correa, T. L. R., Silva, J, C. M., Mansur, L. R. C. O. and Martins, M. L. L., 2008, Properties of an amylase from thermophilic Bacillus sp. Brazilian Journal Microbiology, 39: 102-107.

[14] Cordeiro, C. A. M., Martinas, M. L. L. and Lucaino, A., 2003, Production and properties of alpha amylase from thermophylic Bacillus specie. Brazilian Journal of Microbiology, 33: 13.

[15] Coronado, M. J., Vargas, C., Hofemeister, J., Ventosa, A. and Nieto, J. J., 2000, Production and biochemical characterization of α-amylase from the moderate halophile Halomonas meridian. Literature of Microbiology, 183: 67-71.

[16] Crabb, W., and Mitchinson, E. C., 1997, Enzymes involved in the processing of starch to sugars. Trends Biotechnology, 15(9): 349– 352.

[17] Dettori, C. B. G., Priest, F. G., and Stark, J. R., 1992, Hydrolysis of starch granules by the amylase from Bacillus stearothermophilus NCA 26. Process Biochemistry, 27(1): 17–21.

[18] Deutch, C. E., 2002, Characterization of a salt-tolerant extracellular α-amylase from Bacillus dipsosauri, Literature of Applied Microbiology, 35: 78-84.

[19] Ellaiah, P., Adinarayana, K., Bhavani, Y., Padmaja, P. and Srinivasulu, B., 2002, Optimization of process parameters for glucoamylase production under solid state fermentation by a newly isolated Aspergillus sp. Process Biochemistry, 38: 615–620.

[20] Fisher, E. H. and Stein, E. A., 1961, Alpha amylase from human saliva. Biochemistry Preparation, 8: 27-32.

[21] Fitter, J. and Heberle, J., 2000, Structural equilibrium fluctuations in mesophilic and thermophilic α-amylase. Journal of Biophysics, 79: 1629-1636.

[22] Gangadharan, D., Nampoothiri, K. M., Sivaramakrishnan, S. AND Pandey, A., 2008, Biochemical Characterization of Raw-starch-digesting Alpha Amylase Purified from Bacillus amyloliquefaciens. Applied Biochemistry Biotechnology, 8: 8347-4.

[23] Good, W. M. and Hartman, P. A., 1970, Properties of amylase from Halobacterium halobium, Journal of Bacteriology, 104: 601-603.

[24] Goyal, N., Gupta, J. K. and Soni, S. K., 2005, A novel raw starch digesting thermostable a-amylase from Bacillus sp. I-3 and its use in the direct hydrolysis of raw potato starch. Enzyme Microbial Technology. 37: 723–734.

[25] Guerra, N. P. and Pastrana, L., 2002, Production on mussel-processing waste supplemented with glucose and five nitrogen sources. Literature of Applied Microbiology, 34:114–118.

[26] Haq, I., Ali, S., Javed, M. M., Hameed, U., Saleem, A., Adnan, F. and Qadeer, M. A., 2010, Production of alpha amylase from a randomly induced mutant strain of Bacillus amyloliquefaciens and its application as a desizer in textile industry. Pakistan Journal Botany, 42(1): 473-484.

[27] Haq, I., Ashraf, H., Ali, S. and Qadeer, M. A., 1997, Submerged fermentation of alpha amylase by Bacillus licheniformis GCB-36. Pakistan Biological Society, 43(2): 39-45.

[28] Jogezai, N., Raza, A., Abbas, F., Bajwa, M., Mohammad, D., Kakar, W., Saeed, M and Awan, A., 2011, Optimization of cultural conditions for microbial alpha amylase production. Journal of Microbiology and Antimicrobials, 3(9): 221-227.

[29] Kathleen, T. and Arthur, T., 1996, Foundation in Microbiology. Brown Wm.c. USA, 2- 85.

[30] Kim, D., Moonpark, K. Wooksong, J. and Hoseo, J., 1997, Enhanced production of maltotetraose producing amylase by recombinant Bacillus subtilis LSK 88 in fed batch cultivation. Journal of Microbial Biotechnology, 7(6): 417-422.

[31] Kokab, S., Asghar, M., Rehman, K., Asad, M. J. and Adedyo, O., 2003 Bio-Processing of Banana Peel for α-Amylase production by Bacillus subtilis. International Journal of Agriculture Biology, 5: 1.

[32] Krishnan, T. and Chandra, A. K., 1982, Effect of Oilseed Cakes on α- Amylase Production by Bacillus licheniformis CUMC-305. Applied and Environmental Microbiology, 44: 270- 274.

[33] Kumar, R. A. and Sivasudha, T., 2011, Optimization of Nutritional Constitute for Enhanced Alpha amylase Production Using by Solid State Fermentation Technology. International Journal of Microbiological Research, 2 (2): 143-148.

[34] Kunamneni, A., Permaul, K. and Singh, S., 2005, Amylase Production in Solid State Fermentation by the Thermophilic Fungus Thermomyces lanuginosus. Journal of Bioscience and Bioengineering, 100: 168- 171.

[35] Lealem, F. and Gashe, B. A., 1994, Amylase production by a gram-positive bacterium isolated from fermenting tef (Eragrostis tef). Journal of Applied Bacteriology, 77: 348-352.

[36] Narayana, K. J. P. and Vijayalakshmi, M., 2008, Production of extracellular alpha amylase by Streptomyces albidoflavus. Asian Journal Biochemistry, 3: 194-197.

[37] Ogumentimehin, G. B., 1998, Growth of and amylase production by Bacillus licheniformis isolated from cassava processing waste. Nigerian Food Journal, 11: 58-67.

[38] Oyeleke, S. B. and Oduwole, A. A., 2009, Production of amylase by bacteria isolated from a cassava waste dumpsite. African Journal Microbiology, 3(4): 143-146.

[39] Omori, T., Takeshima, N. and Shimoda, M., 1994, Formation of acid labile alpha amylase during barley Koji production. Journal of Fermentation Technology Bioengineering, 78(1); 27-30.

[40] Onishi, H. and Sonada, K., 1979, Purification and some properties of an extracellular amylase from moderate halophile, Micrococcus halobius, Applied Environmental Microbiology, 38: 616-20.

[41] Oyeleke, S. B., Auta, S. H., and Egwim, E. C., 2010, Production and characterization of amyalse production by Bacillus megaterium isolated from local yam peel. Journal of Microbiolgy and Antimicrobials, 2(7), 88-92.

[42] Pandey, A., Nigam, P., Soccol, C.R., Soccol, V.T., Singh, D. and Mohan, R., 2000, Advances in microbial amylases. Biotechnology Applied Biochemistry, 31(2): 135-152.

[43] Rajagopalan, G. and Krishnan, C., 2008, Alpha-amylase production from catabolite derepressed Bacillus subtilis KCC103 utilizing sugarcane bagasse hydrolysate. Bioresource Technology, 99: 3044- 3050.

[44] Ramesh, M. V. and Lonsane, B. K., 1991, Ability of solid state fermentation technique to significantly minimise catabolic repression of alpha amylase production by Bacillus licheniformis M 26. Applied Microbiology Biotechnology, 35 (5): 591-593.

[45] Rasiah, I. A. and Rehm, B. H., 2009, One-step production of immobilized alpha-amylase in recombinant Escherichia coli. Applied Environmental Microbiology, 75 (7): 2012-6.

[46] Rao, M. B., Tanksale, A. M. and Gathe, M. S., 1998, Molecular and Biotechnological aspects of microbial proteases. Microbial Molecular Biology Revised, 62 (3): 597-635.

[47] Riaz, N., Haq, I. and Qadeer, M. A. 2003, Characterization of α-Amylase by Bacillus subtilis. International Journal of Agriculture & Biology, 3: 249–252.

[48] Roohi, K. M., Ahmad, I. Z. and Arif, J. M., 2011, Production of cold-active extracellular α-amylase by newly isolated Microbacterium foliorum GA2. Asian Journal Biotechnology, 3(5): 449-459.

[49] Sajedi, R. H., Naderi M. H., Khajeh, K., Ahmadvand, R., Ranjbar, B., Asoodeh, A. and Moradian, F. 2005, A Ca-independent α-amylase that is active and stable at low pH from the Bacillus sp. KR-8104. Enzyme Microbiology Technology, 36: 666-671.

[50] Santos, E. O. and Martins, M. L. L., 2003, Effect of the medium composition on formation of amylase by Bacillus sp. Brazilian Achieves Biology Technology, 46: 129.

[51] Shafaat, S., Akram, M. and Rehman, A., 2011, Isolation and characterization of a thermostable α-amylase from Bacillus subtilis. African Journal of Microbiology Research, 5(20):3334-3338.

[52] Sidhu, G. S., Sharma, P., Chakrabarti, T. and Gupta, J. K., 1997, Strain improvement for the production of a thermostable alpha-amylase. Enzyme Microbial Technology, 24:584-589.

[53] Srivastava, R. A. K, and Baruah, N., 1986, Culture conditions for production of thermo stable amylase by Bacillus stearothermophilus. Applied and Environmental Microbiology, 52: 179-184.

[54] Teodoro, S. and Martins, L. 2000, Culture conditions for the production of thermostable amylase by Bacillus sp. Brazilian Journal Microbiology, 31: 298-302.

[55] Thippeswamy, S., Girigowda, K. and Mulimani, V. H., 2006, Isolation and identification of alpha amylase producing Bacillus sp., from dhal industry waste. Indian Journal of Biochemistry and Biophysics, 43: 295-298.

[56] Vanova, V., Yankov, D., Kabaivanova, L. and Pashkoulov, D., 2001, Simultaneous biosynthesis and purification of two extracellular Bacillus hydrolases in aqueous two alpha amylase. Journal of Biochemical Engineering, 8(1): 61-81.

[57] Varalakshmi, K. N., Kumudini, B. S, Nandini, B. N., Solomon, J. D., Mahesh, B., Suhas, Vaseekaran, S., Balakumar, S. and Arasaratnam, V., 2010, Isolation and Identification of a Bacterial Strain Producing Thermostable α- Amylase from different sources such as soil, gruel of rice and bakery waste. Applied Biochemistry Biotechnology, 22 (1): 1-11.

[58] Vidyalakshmi, R., Paranthaman, R. and Indhumathi, J., 2009, Amylase Production on Submerged Fermentation by Bacillus spp. World Journal of Chemistry, 4 (1): 89-91.

[59] Vijayabaskar, P., Jayalakshmi, D. and Shankar, T., 2012, Amylase production by moderately halophilic Bacillus cereus in solid state fermentation. African Journal of Microbiology Research, 6(23): 4918-4926.