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Studies on Optimization of Amylase Enzyme Isolated from Bacillus spp.

Microbiology

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ABSTRACT

The enzyme from microbial sources are more stable and obtained cheaply. Amylases are among the most important enzyme and are of great significance in present day industry. Bacterial amylase from isolate Ak-3 (Bacillus subtilis) was potential source for industrial exploitation in the field of amylase production. Hence, this study on bacterial amylase was done to found optimum kinetic parameters, which increases the enzyme activity. The optimum conditions of an α-amylase activity were experimentally investigated in details. The optimum activity was achieved with 1.0 concentration unit of α-amylase and 1.0% starch concentration in phosphate buffer solution pH 7 at 37°C for 30 mins. The amylase activity was monitored by dinitrosalicylic acid method based on the measurement of maltose. The maximum activity was recorded at pH 7.0, the optimum temperature was found to be 37°C. The metal ion CuSO4 shows maximum activity. The maximum activity was recorded at 0.9-1 ml substrate concentration. The optimum time course for amylase activity was found to be at 30 mins. and optimum enzyme concentration was recorded at 0.9-1 ml concentration. Among various C and N sources, 0.5% sucrose and 1% Ammonium sulphate showed maximum activity. 0.1% EDTA concentration was found to be optimized for amylase production. The further detail study could benifit for amylase production in industries from the isolate.

KEYWORDS:

Amylase, Bacillus subtilis, EDTA.

Introduction:-

Amylases are obtained from various origins like plant, animal, bacterial and fungal. Several researchers produces amylase enzyme using Bacillus sp. There are about 3000 enzymes known today only few are industrially exploited. There are mainly extracellular hydrolytic enzymes, which degrade naturally occurring polymers such as starch, proteins, pectin's and cellulose. The industrial enzyme producers sell enzymes for a wide variety of applications. The estimated value of world market is presently about US$ 2.7 billion and is estimated to increase by 4% annually through 2012 (Panneerselvam and Elavarasi, 2015).

α-Amylase with suitable properties can be very useful in a specific industry, thus it has become essential to characterize all available microbial strains for their productivity. Since almost all microorganisms of the Bacillus genus synthesis α-amylase, this genus has the potential to dominate the enzyme industry. Bacillus species are heterogeneous forms of organisms and they are very versatile in their adaptability to the environment (Bozic et al., 2011).

α-Amylases have potential application in a wide number of industrial processes such as food, fermentation, textile, paper, detergent and pharmaceutical industries. However, with the advances in biotechnology, the amylase application has expanded in many fields such as clinical, medicinal and analytical chemistry, as well as their widespread application in starch saccharification and in the textile, food, brewing and distilling industries (De Souza and Magalhaes, 2010). Thus, the present study has undertaken to study the optimization of amylase from the selected isolate.

Material and Methods:-

A) Production of Amylase:

The isolate Ak-3 that showed prominent amylase activity on starch agar medium was used for inoculums preparation. The culture medium containing soluble starch (1% in 100ml), peptone, Meat extract, sodium chloride, in this culture medium then inoculate 24 hrs. old culture. The flask were incubated at 37°C for 3 days i.e 72 hrs. in a shaking incubator (120 rpm). The culture broth was then centrifuged at 5,000 rpm for 20 mins, the cell free supernatant was used as an extracellular crude enzyme amylase for further tests.

B) Protein assay:

The concentration of protein was determined by Lowry's method using bovine serum albumin as the standard.

C) Enzyme assay:

Enzyme was assayed to determine through the reducing sugar by the dinitrosalicylic method. The reaction mixture containing of 1% substrate in 0.1M phosphate buffer, it was kept for 20mins in incubator at 37°C for activation of enzyme then enzyme was added and again kept it in incubator for 30 mins at 37°C for incubation. The reaction was stopped by adding 1ml of 3,5-dinitrosalicylic acid solution followed by heating in a boiling water bath for 10 min., the level is maintained up to 12 ml. Absorbance of each solutions was measured at 540nm by Spectrophotometer.

D) Effect of various parameters (Temperature, pH, Substrate conc., Time course, Metal ion, Enzyme conc.):-

The optimization was carried by using enzyme extract to study the effect of temperature, pH, substrate concentration, metal ion, time course and enzyme concentration. For temperature optimization the temperature optimum of the enzyme was evaluated by measuring the amylase activity at different temperatures (5-80°C) and the enzyme extract was incubated at different temperatures for 30 min. The activity was checked using DNSA method. The optimum pH of the enzyme was determined by varying the pH of the assay reaction mixture using the different pH (3.6-8), the pH is maintained by adding NaOH and HCl. The optimum pH was found by DNSA method by keeping it for 30 mins. The substrate concentration was carried out by adding different concentration from 0.1-1ml. The time course is very important for the activity of enzyme so the enzyme was kept for different time (5-50 min) and then optimum time was determined by assay method. The enzyme activity was studied using different metal ions. viz. ammonium chloride, ammonium sulphate and sodium nitrate. The enzyme concentration was determined by using different enzyme concentration from 0.1-1 ml.

In all the parameters the substrate, phosphate buffer, crude enzyme are added at required quantity then it was kept in the incubator for 30

min. Then DNSA reagent was added 1ml in each tube and then determined by spectrophotometer at 540 nm.

Results:-

Generally, the optimization study of any enzymatic activity is carried out by a enzyme assay. In this case, five parameters affecting the amylase activity including an enzyme and starch concentration, pH of the solution, incubation time, temperature and metal ions were investigated in detail. In the study the isolate Ak-3 (Bacillus spp.) previously isolated was used which showed excellent activity in the screening study. After production of the amylase in the medium the cell free supernatant was separated by centrifugation at 5000 rpm for 20 min and used as crude enzyme. Then different optimization parameters were studied.

- **Protein estimation:-**

Protein concentration from the crude enzyme sample was determinate by Folin-lowry method. The protein content was found in the cell free supernatant of isolate Ak-3 that was 104 µg/ml.

- **Enzyme assay:-**

Amylase concentration from the crude enzyme sample was determined by Sumner's method. The standard graph of maltose was plotted. From the standard graph the activity of crude enzyme was calculated and isolate Ak-3 showed activity which was 42 U/ml.

- **Effect of various parameters on enzyme activity of amylase:**

- **Temperature:** The effect of temperature was studied from 5°C to 80°C. The optimum temperature of amylase produced from the isolate Ak-3 was found to be at 37°C. The result of other temperature are shown in graph Fig.3.

- **pH :** The assay of isolate Ak-3 was done at pH 3.6 to 8 using different buffers. Highest activity of amylase was found to be at pH 7 whereas lowest activity was at pH 3.6 and 8.

- **Substrate concentration:** The medium was centrifuged and the supernatant was collected for amylase assay. The assay was done at different concentration from 0.1 to 1ml. Higest activity of amylase was found to be at 0.9-1ml and then its levels off.

- **Metal ion:** The assay was done by using different metal ions like FeCl3, BaCl2, MgSO4, CuSO4. Highest activity of amylase was found by CuSO4 whereas lowest activity was noted after addition of BaCl2 metal ion.

- **Time Course :** The assay was done at different time intervals from 5 to 50 mins. The highest activity was found at 30 min whereas lowest activity was noted at 5 min. The other result was shown in Fig 7.

- **Enzyme concentration:** The isolate Ak-3 was inoculated in production medium for 72 hrs at 37°C in a shaker. The medium was centrifuged and the supernatant was collected for amylase assay. Assay was done at different enzyme concentrations from 0.1 to 1ml. The result are shown in Fig 8.

- **Effect of various carbon and nitrogen sources on enzyme production :**

- **Effect of carbon sources:**

Among the various carbon sources used for amylase production, sucrose was found to be the best substrate, showing maximum enzyme activity of 4.5 U/ml. The other results of glucose and maltose are shown in Fig 9.

Effect of nitrogen sources:

Effect of various nitrogen sources on amylase production in growth medium was also examined. It was observed that growth medium containing 1% ammonium sulphate produced maximum amylase. This was followed by ammonium chloride and sodium nitrate (fig 10).

- **Effect of EDTA concentration on enzyme:**

The isolate Ak-3 was inoculated in production medium with different EDTA concentrations for 72 hrs at 37°C in a shaker. Then medium was centrifuged and the supernatant was collected for amylase assay. Amylase activity was found high in 0.1% EDTA containing solution.

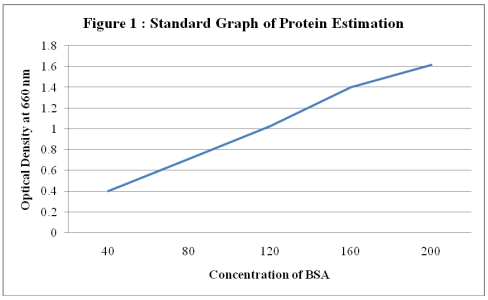


Table No. 1: Protein estimation in crude enzyme of isolates Ak-3.

Sr. No.	Isolate	Optical Density at 660 nm	Protein conc. (µg/ml)
1	AK-3	0.92	104

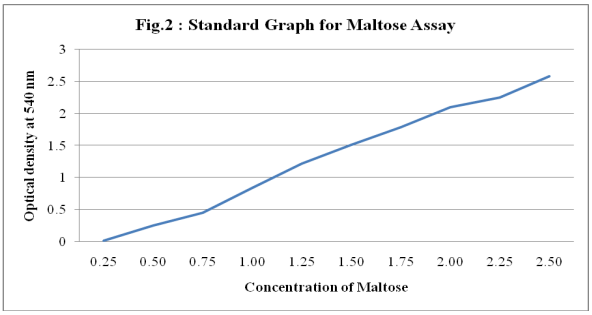
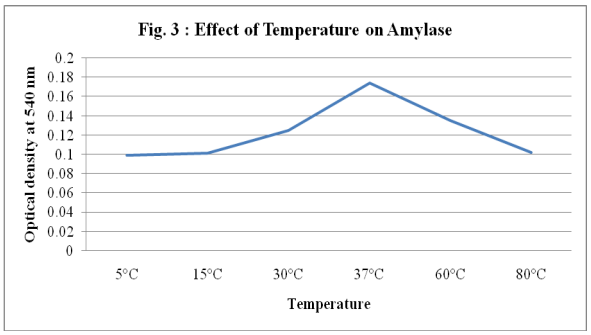
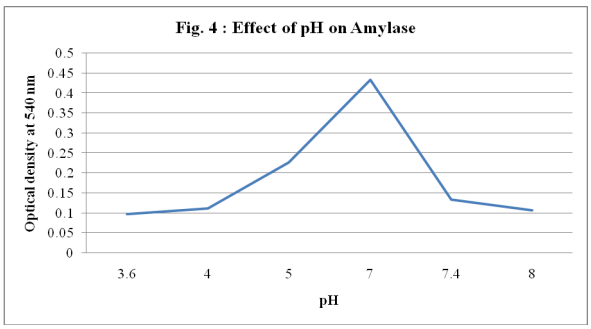
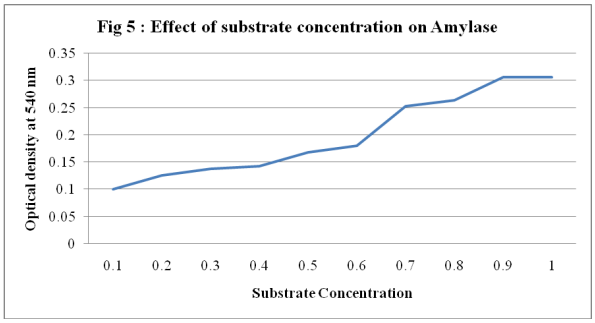


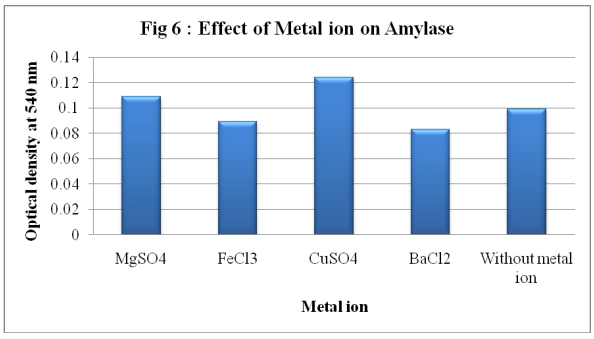
Table No.2: Enzyme Assay for crude enzyme from isolate Ak-3

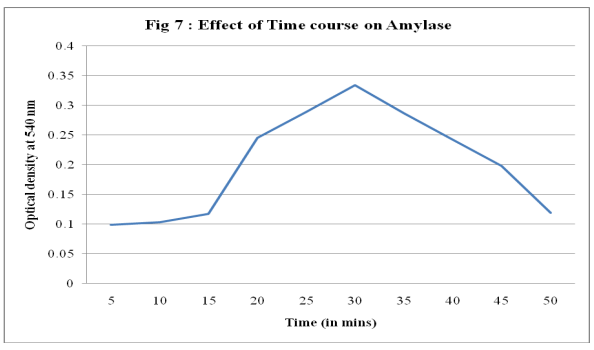
Sr. No.	Isolate	Optical density at 540 nm	Activity (U/ml)
1	AK-3	1.403	42

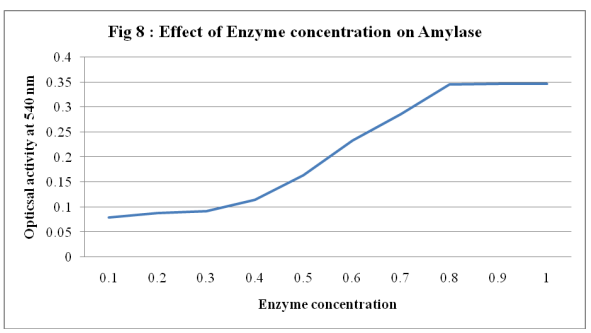


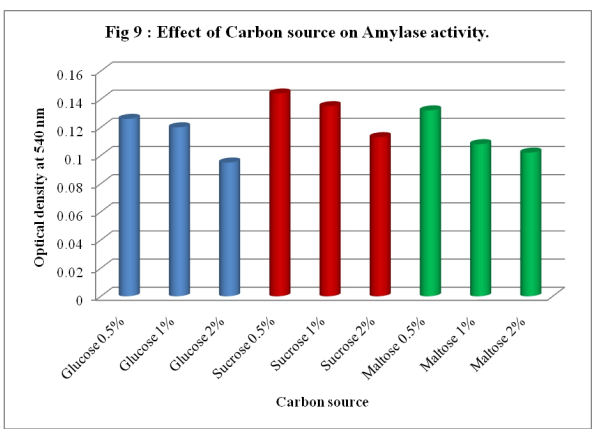


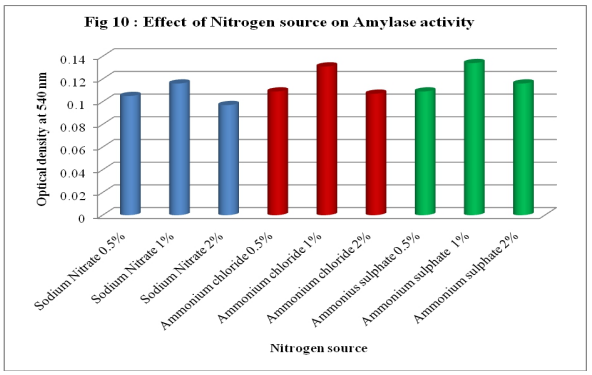


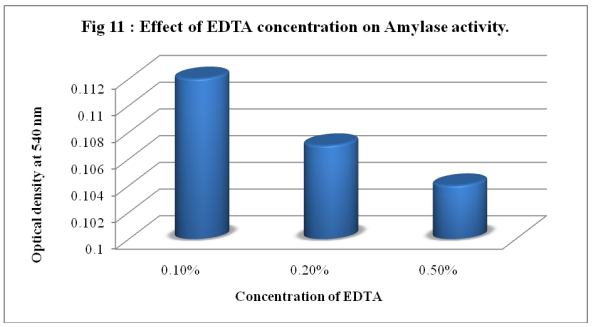












Discussion:

Amylases are among the most important enzymes and are of great significance for biotechnology, constituting a class of industrial enzymes having approximately 25% of the world enzyme market.

In study, the isolate was subjected to amylase production by shake flask fermentation containing required medium. After incubation the cell free supernatant containing crude enzyme was checked for protein content by Folin Lowry method. The protein concentration obtained was 104 µg/ml. It was higher concentration than other studies Paul et al., (2016) reported 3.1 mg/ml protein concentration. The activity of enzyme was determined by enzyme assay by dinitrosalicylic acid method. The enzyme activity was 42 U/ml obtained with isolate Ak-3. To this findings some studies are in concordance, they reported more enzyme activity from crude enzyme extract which was 764.68 U/ml by Paul et al., (2016).

In the present study the production medium for bacterial enzyme activity was produced by shake flask fermentation at 37°C incubated for 72 hrs. The cell free supernatant was removed by centrifuged at 5000 rpm for 20 min. This supernatant was used as crude enzyme source. Enzyme assay was done by dinitrosalicylic acid method at 540 nm.

In the study, it was found that the crude enzyme for isolate Ak-3 were showing maximum activity at 37°C. This observation was similar with the Viswanathan et al., (2014) they reported enzyme activity was maximum at 37°C, Poddar et al., (2012) also reported maximum activity at 37°C for Bacillus subtilis. This could be due to the mesophilic nature of the organism. This finding is in concordance with the other studies as most of the studies reported activity of enzyme was more at high temperature. Vishnu et al., (2014), Verma et al., (2011) reported amylase from Bacillus spp was showing maximum activity at 40°C, Kanimozhi et al., (2014) found maximum activity at 50°C. Some studies reported less temperature for maximum activity of amylase from Bacillus spp. like Alariya et al., (2013) reported 35°C optimum temperature for enzyme activity. This reports are supports our findings.

The isolate AK-3 showed maximum activity at pH 7 after 72 hrs (fig. 4). This is in agreement with other studies who also reported pH7 was optimum for amylase activity of Bacillus spp. (Viswanathan et al., 2014, Haribhau et al., 2015, Padhiar & Kommu, 2016, De souza and

Magalhaes, 2010). Some studies are in concordance to this finding and they reported maximum enzyme activity at alkaline pH like Verma et al., (2011) found optimum pH were 7.5 and 8.5 for Bacillus spp. and Kanimozhi et al., (2014) reported highest activity at pH 8.

Starch concentration results showed that starch at a concentration of 0.9-1 ml had maximum enzyme activity for isolate Ak-3 because after that it levels off. Similar to this swain et al., (2006) also reported 1% starch concentration was optimal for enzyme activity, followed they found decline at higher concentrations. Concordance to this findings Padhiar & Kommu, (2016) reported more enzyme activity on 5% starch concentration for Pseudomonas mendocina and 2% substrate concentration for Bacillus subtilis and Poddar et al., (2012) reported maximum activity at concentration 5 mg/ml for Bacillus spp.

Different metal ions (FeCl3, BaCl2, MgSO4, CuSO4) have been tested for amylase production using crude enzyme of isolate AK-3. Maximum activity of 3.9 U/ml of amylase was determined in presence of CuSO4 ion and was shown in fig.6. Opposite to this Padhair & Kommu, 2016 reported maximum activity in presence of Mg ion, similar to this in this study addition of Mg ion also showed increased activity but activity at 2nd position. Similarly Arfah et al., (2015) reported Mg ion shows highest activity at 2nd position and at 1st position Ca ion showed highest activity. According to Naidu and saranraj, 2013 they reported the presence of Zn, Na, Na-sulphide inhibite the enzyme production.

Incubation period plays important role in the production of enzyme. The period may varies. The optimum time for enzyme activity in the study showed the crude enzyme from isolate AK-3 shows optimum activity at 30 mins in (fig.7), similar to present study Paul et al., (2016) also reported 30 mins of incubation time was suitable for proper enzyme activity.

The isolate AK-3 showed maximum amylase production in the presence 0.5% concentration of sucrose and then maltose at 0.5% concentration. This findings are in concordance to Alariya et al., 2013 who reported 0.5% concentration showed maximum activity that the different carbon sources have varied influence on the extracellular enzymes especially amylase strains. Similar to this Rao and Sathyanarayana, (2003) reported that the different carbon sources have varied influence on the extracellular enzymes especially amylase strains. Results obtained showed that increase in concentration of various substrates increases the amylase production more in glucose. (Padhiar & Kommu, 2016) observed more activity at 1% glucose. Shalinimol, (2016) reported maximum activity in maltose and second highest activity in sucrose.

The amylase production by the selected isolate was also optimized by supplementing different inorganic nitrogen sources individually at the concentration of 0.5% to 2% such as ammonium sulphate, ammonium chloride and sodium nitrate. Among inorganic nitrogen sources ammonium sulphate was found to be best. The maximum activity was shown by ammonium sulphate at 1% concentration. This finding was similar to Padhiar and Kommu, (2016) , Shalinimol, (2016) who also reported maximum enzyme activity from ammonium sulphate. This is in concordance to the findings of Alariya et al., (2013) who reported maximum activity by sodium nitrate for Bacillus spp. and Serratia marscens.

Influence of various concentration of EDTA on amylase production was determined by incubating the medium with different concentration from 0.1 to 0.5% to medium. The maximum enzyme activity was observed maximum for 0.1% concentration of EDTA. This is in concordance to the findings of the Alariya et al., (2013), who reported maximum activity at 0.2% concentration of EDTA and again increase at 0.5%.

References:-

1. Alariya S, Sethi S, Gupta S and Gupta B, (2013): Amylase activity of a starch degrading bacteria isolated from soil. Scholars Research Library Archives of Applied Science Research., 5 (1) : 15-24.
2. Arfah R. A, Ahmad. A, Djide M. N, Anis M, Zakir M, (2015): Production Optimization and Characterization of Amylase Enzyme Isolated from Termofil Bacteria Bacillus sp. RSAII-1b Lejja Hot Spring South Sulawesi. American Journal of Biomedical and Life sciences, 3 (6) : 115-119.
3. Bozic N, Ruiz J, Santin L. J and Vujcic Z, (2011): Optimization of the growth and -amylase production of Bacillus subtilis IP 5832 in shake flask and Laboratory fermenter batch cultures. Journal of Serbian Chemical Society. 76 (7) 965-972.
4. De souza P. M, and Magalhaes P, (2010): Application of Microbial -amylase in industry- A Review. Erazilian Journal of Microbiology. 41 : 850-861.
5. Haribhau K, Patil V. B and Dr. Patil A. P, (2015): Isolation, Screening and Optimization of Microorganism Producing Amylase. World Journal of Pharmacy and Pharmaceutical sciences. 4 (7) 1415-1425.
6. Kanimozhi M, Johny M, Gayathri N, Subashkumar R, (2014): Optimization and Production of -amylase from Halophilic Bacillus species. Isolated from Mangrove soil sources. Journal of Applied and Environmental Microbiology. 2 (3) 70-73.
7. Naidu and Saranraj, (2013): Bacterial Amylase : A Review. International journal of Pharmaceutical and Biological Archives. 4 (2) : 274-287.
8. Paul J. S, Lall B. M, Jadhav S. K, Tiwari K. L, (2016): Parameters optimization and kinetics study of -amylase enzyme of Bacillus sp. MB6 isolated from vegetable waste. Process Biochemistry : 1-7.
9. Poddar A, Gachhui R, Jana S. C, (2012): Optimization of physic-chemical condition for improved production of hyperthermostable -amylase from Bacillus subtilis DJ5. J. Biochem. Tech. 3 (4) : 370-374.
10. Panneerselvam T and Elavarasi S, (2015): Isolation of -amylase producing Bacillus subtilis from soil. Internstional Journal of Current Microbiology and Applied Sciences. 4 (2) : 543-552.
11. Padhiar A. R, and Kommu S, (2016): Isolation, Characterization and Optimization of bacteria producing amylase. International Journal of Advanced Research in Biological Sciences. 3 (7) : 1-7.
12. Rao J. L and Sathyanarayana T, (2003): Enhanced secretion and low temperature stabilization of a hyperthermostable and Ca2+ independent alpha-amylase of geo Bacillus thermoleovorans by surfactants. Lett. Appl. Microbiol. 36 : 191-196.
13. Shalinimol C. R, (2016): A study on optimization of Microbial Alpha-amylase production. DJ International Journal of Advances in Microbiology and Microbiological Research. 1 (1) : 22-27.
14. Verma V, Avasthi M, Gupta A, Singh M and Kushwaha A, (2011): Amylase production and purification from bacteria isolated from a waste potato dumpsite in district Farrukhabad U.P. State India. Pelagia Research Library European Journal of Experimental Biology. 1 (3) : 107- 113.
15. Viswanathan S, Rohini S, Rajesh R and Poomari K, (2014): Production and medium optimization of amylase by Bacillus spp. using submerged fermentation method. World Journal of Chemistry. 9 (1) : 01-06.
16. Vishnu T. S, Soniyamby A. R, Praveesh B. V, Hema T. A, (2014): Production and optimization of extracellular amylase from soil receiving kitchen waste isolate Bacillus sp. VS 04. World Applied Sciences Journal. 29 (7) : 961- 967.