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# Isolation, production and characterization of enzyme amylase produced from bacteria isolated from bat guano of Lonar Lake

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**Abstract**
An alkaline Lonar Lake is a nature's gift to human being. It is an unique Basaltic rock meteorite impact crater present on the earth. The surrounding ecosystem of Lonar crater is responsible for the occurrence of rare and unique life forms. The pH of Lonar lake crater ranges between 10-12 due to the combination of geological, geographical and climatic conditions. Surrounding the lake region there is a presence of various temples which are in deteriorated conditions and away from the attention. Such temples are the dwelling places of bats and monkeys present at Lake region. Bats are mainly dependent upon Lonar lake and its surrounding for its food and water purpose. So such bats which are dependent upon alkaline water of Lonar lake strongly having the chances of production of such enzymes (Amylase, Protease, Lipase, etc.) which are stable at high alkaline pH and so such study about the production and characterization of enzymes from bat guano of Lonar lake is having its great importance regarding to industries such as cloth, paper, detergent, etc

**Keywords:** Alkaline, basalt rock, temples, bat guano, enzymes

## Introduction

### Lonar Lake

Extremophiles are the microorganisms that can grow and thrive in extreme environments, which were formerly considered too hostile to support life. The extreme conditions may be high or low temperature, high or low pH, high salinity, high metal concentrations, very low nutrient content, very low water activity, high radiation, high pressure and low oxygen tension. Some extremophiles are subject to multiple stress conditions. Extremophiles are structurally adapted at the molecular level to withstand these harsh conditions (Gomes and Steiner, 2004) [7]. This alkaline Lonar Lake is a unique basaltic rock meteorite impact crater ranking third in the world and established before 50,000 yrs ago. This Lonar lake has periphery of 1.7 km. and situated in a hallow 0.14km.below the ground level and amphitheatre of vertical cliff. The lake is circular except on the north-eastern side, where siltation caused by Dhar has created small mud flats. The diameter of the crater at the surface is about 1300m. The lake absolutely confined from all sides by the walls of the crater and there is not a single channel of water draining away from it. The presence of the crater is responsible for generating more ecological variables, within small localized system that could be expected in such semiarid area otherwise. An important ecological principle is that, greater the complexity of ecological variables, greater is the potential and possibility of rare and unique life forms being supported by the ecosystem. In the case of Lonar this principle stands validated. Soda lakes are the most stable and productive naturally occurring alkaline environments in the world, with pH values generally greater than 10 and occasionally reaching 12.

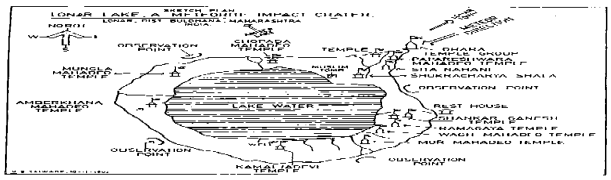
These alkaline environments are caused by the combination of geological, geographical and climatic conditions. They are characterized by large amounts of sodium carbonate formed by evaporative concentration. In the course of formation of alkalinity, other salts particularly (NaCl) also concentrate, giving rise to an alkaline
saline environment (Grant *et al*, 1990) [9]. Various workers have worked on the various contents of Lonar Lake such as carbonates, bicarbonates, chlorides, sodium, potassium, etc. The carbonate and bicarbonate falls in the range of 1494mg/L and 1413mg/L respectively. Similarly, nitrate shows 8mg/L and phosphate content is 1.3mg/L (Mahajan AD, 2005).

## Materials and Method

| Contents | Medium A | Medium C |
|---|---|---|
| Glucose | 10 | _ |
| Soluble | _ | _ |
| peptone | 5 | 5 |
| yeast extract | 5 | 1.5 |
| beef extract | _ | 1.5 |
| NaCl | _ | 5 |
| $KH_2PO_4$ | 1 | _ |
| $MgSO_4.7H_2O$ | 0.2 | _ |
| $Na_2CO_3$ | 10 | _ |
| Agar | 20 | 20 |
| pH | 9 | 10 |

**Reagents:** Crystal violet, Gram's iodine, Alcohol, Saffranine, Phosphate buffer, 1% Starch, 1 % NaCl, DNS+NaOH,NaKtartarate,Enzyme,HydrogenPeroxide,NNNNTetramethylParaphenlylenediaminedihydrochloride,Lactose,Dextrose, Mannitol, Sucrose and Fructose

**Collection of sample:** Eight bat guano samples were collected from Ramgaya temple, Wagh Mahadeo temple and Kamalja Devi surrounding the lake region. The samples were collected with the help of scooper in sterile polyethylene bags. The samples were labeled as per the spot, time and date.




**A Sketch plan of Lonar Lake, a meteorite impact crater**

The suspension of bat guano sample was done by taking 1g of sample in 9mL distilled water and streak plate method was applied. The pH of the medium was adjusted to 10-10.5 by using 1N NaOH. Two media were used for the cultivation of bacteria from the bat guano samples.

### Preparation of Suspension of Bat Guano Samples

One gram from each Bat Guano sample was dissolved in 9 mL of sterile distilled water and makes the suspension for inoculation on media.

### Isolation of bacterial isolates

These bat guano samples were then inoculated by streak plate method on A and C media. Isolated colonies were examined for colony character. Then these pure isolated colonies were cultivated on media C. These pure cultures were then stored at 4 $^0$C and maintain as a stock cultures.

### Preparation of sub culture

Sub culture slants were prepared by using 'C' medium (pH 10.5). Then sub cultures were prepared from stock cultures. From these subcultures pure broth cultures were prepared which were confirmed by performing Gram staining, motility, endospore staining and various biochemical tests. These pure isolates obtained from bat guano samples were analyzed for hydrolysis of Starch.

### Screening of amylase producing bacteria

Isolates which we have isolated and broth cultured were checked for amylase activity on starch agar 1%. These isolated were inoculated on alkaline starch agar of pH 10 by point inoculation. Plates were incubated at 37 $^0$C for 72h and then zone of starch hydrolysis.

### Identification of amylase producing bacteria

The organisms which produced zone of starch hydrolysis on alkaline starch agar medium were identified on the basis of their morphology, Gram reaction, motility and other biochemical, and cultural characteristics.

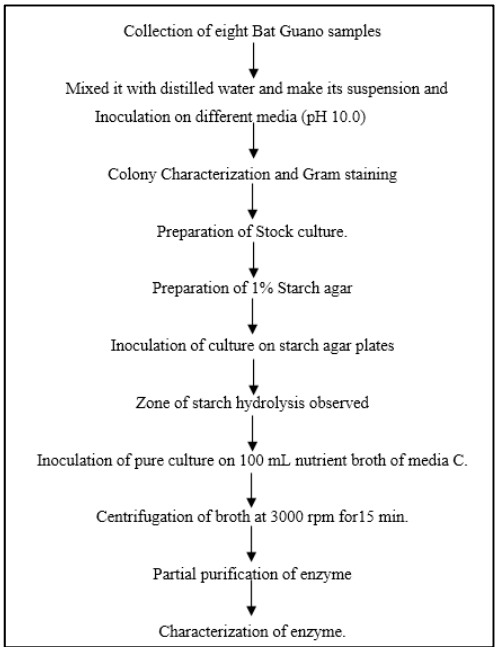
### Production of enzyme

The isolated pure amylase producing organism was inoculated in the broth medium of media C having pH 10 and were incubated for 24- 72 h. After 24- 72 h of incubation, crude enzyme production was observed in the medium. The crude amylase enzyme was then partially purified by adding Ammonium Sulphate in the medium containing enzyme amylase.

### Characterization of enzyme

The characterization of obtained enzyme amylase was seen by its activity against various parameters like pH, Temperature, Enzyme Concentration and Substrate concentration.

### Method for analysis of bat guano sample



**Protocol for effect of Temperature on enzyme activity**

| Sr. No | Reagents | Blank | Control | 40 | 50 | 60 | 70 | 80 | 90 |
|---|---|---|---|---|---|---|---|---|---|
| 1 | $PO_4$buffer | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 |
| 2 | starch | _ | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 |
| 3 | 1% NaCl | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Keep all tubes at 10min. for different temperature. | | | | | | | | | |
| 5 | DNS+NaOH | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Keep all tubes for 5min. in boiling water bath | | | | | | | | | |
| 6 | Na-K tartarate | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 7 | D. Water | 7.5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| Take O.D. readings at 540nm | | | | | | | | | |

**Protocol for effect of pH on enzyme activity**

| Sr. No. | Reagents | blank | Control | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | pH | 6.7 | 6.7 | 6 | 7 | 7.5 | 8 | 8.5 | 9 | 9.5 | 10 |
| 2 | $PO_4$ buffer | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 |
| 3 | starch | - | - | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 |
| 4 | 1% NaCl | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 5 | Enzyme | - | - | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Incubate all tubes at 37c for10 min | | | | | | | | | | | |
| 6 | DNS+NaOH | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Keep all tubes in boiling water-bath for 5min. | | | | | | | | | | | |
| 7 | Na-K tartarate | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 8 | D. water | 7.5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| Take O.D. readings at 540 nm. | | | | | | | | | | | |

**Protocol for effect of Substrate Concentration on enzyme activity**

| Sr. No. | Reagents | blank | Control | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 2 | $PO_4$ buffer | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 |
| 3 | starch | - | - | 0.5 | 1 | 1.5 | 2 | 2.5 | 3 | 3.5 | 4 |
| 4 | 1% NaCl | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 5 | Enzyme | - | - | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Incubate all tubes at 37c for10 min | | | | | | | | | | | |
| 6 | DNS+NaOH | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Keep all tubes in boiling water-bath for 5min. | | | | | | | | | | | |
| 7 | Na-K tartarate | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 8 | D. water | 7.5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| Take O.D. readings at 540 nm. | | | | | | | | | | | |

**Protocol for effect of Enzyme Concentration on enzyme activity**

| Sr. No. | Reagents | blank | Control | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 2 | $PO_4$ buffer | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 |
| 3 | starch | - | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 |
| 4 | 1% NaCl | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 5 | Enzyme | - | - | 0.5 | 1 | 1.5 | 2 | 2.5 | 3 | 3.5 | 4 |
| Incubate all tubes at 37c for10 min | | | | | | | | | | | |
| 6 | DNS+NaOH | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Keep all tubes in boiling water-bath for 5min. | | | | | | | | | | | |
| 7 | Na-K tartarate | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 8 | D. water | 7.5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| Take O.D. readings at 540 nm. | | | | | | | | | | | |

## Results and Discussion

The morphological and biochemical characteristics of 13 isolates were studied according to Bergey's Manual of Systemic Bacteriology. Identification of isolates was carried out on the basis of cultural, morphological and biochemical characteristics. Organism species such as *Enterococcus, Micrococcus, Alkaligens, etc* were identified by performing morphological and biochemical tests. The following results were shown by the identified microbial species isolated from the bat guano of Lonar Lake.

**Characterizations of bacteria isolated from bat guano**

| Sr. No. | Culture Code | Media | Source | Colony Shape | Color of Colony | Gm Staining | Shape | Arrangement | Spore | Motility | Catalase | Oxidase | Lactose | Dextrose | Mannitol | Fructose | Sucrose | Amylase | Protease | Lipase | Name of Microorganism |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 3a | C | BG | Circular | Colorless | + | C | P | - | - | - | + | + | - | - | - | - | - | + | + | *Enterococcus durans* |
| 2 | 4a | C | BG | Circular | Colorless | - | SR | S | + | + | - | + | + | + | + | + | + | + | - | + | *Bacillus sp.* |
| 3 | 5a | C | BG | Circular | Yellow | + | C | CL | - | - | - | - | + | + | - | - | - | - | + | + | *Micrococcus varians* |
| 4 | 9a | C | BG | Circular | Colorless | - | SR | P | + | - | - | + | - | - | + | - | - | + | - | + | *Bacillus sp.* |
| 5 | 10a | C | BG | Circular | Colorless | + | C | CL | - | - | + | - | + | - | - | - | - | - | + | - | *Pediococcus halophilus* |
| 6 | 19a | C | BG | Circular | Colorless | + | C | CH | - | - | - | + | + | - | + | - | - | - | + | + | *Enterococcus faecium* |
| 7 | 20a | C | BG | Circular | Colorless | - | LR | S | + | + | + | + | - | - | - | + | - | + | - | - | *Alkaligens sp.* |
| 8 | 22a | C | BG | Circular | White | + | LR | S | - | + | + | + | + | + | + | + | + | + | - | - | *Bacillus sp.* |
| 9 | 24a | C | BG | Circular | Colorless | + | SR | S | + | - | - | + | - | - | + | - | - | + | - | + | *Bacillus sp.* |
| 10 | 9 | C | BG | Circular | White | + | C | P | - | - | + | - | - | - | - | - | - | - | - | + | *Planococcus kocurii* |
| 11 | 10 | C | BG | Circular | White | + | C | P | - | - | + | - | - | - | - | - | - | - | - | + | *Planococcus kocurii* |
| 12 | 11 | C | BG | Circular | Colorless | + | C | CH | - | - | + | + | - | - | - | - | - | - | - | + | *Micrococcus agilis* |
| 13 | 12 | C | BG | Circular | Colorless | + | C | CL | - | - | + | - | + | - | - | - | - | - | + | - | *Pediococcus halophilus* |

(Where BG= bat guano, C= Cocci, SR= Short Rod, LR= Long Rod, P= Pair, S= Single, CL= Cluster, CH= Chain)

**Identification of isolates on the basis of Gram staining, Motility and Spore formation**

| Sr. No. | Sample Code | Gram Reaction | Motility | Spore |
|---|---|---|---|---|
| 1 | 3a | + | - | - |
| 2 | 4a | - | + | + |
| 3 | 5a | + | - | - |
| 4 | 9a | - | - | + |
| 5 | 10a | + | - | - |
| 6 | 19a | + | - | - |
| 7 | 20a | - | + | + |
| 8 | 22a | + | + | - |
| 9 | 24a | + | - | + |
| 10 | 9 | + | - | - |
| 11 | 10 | + | - | - |
| 12 | 11 | + | - | - |
| 13 | 12 | + | - | - |

From bat guano samples of Lonar Lake13 isolates were isolated. Identification of isolates was based on cultural, morphological and biochemical characteristics. Out of 13 isolates 9 were found to be Gram positive while 4 were Gram negative, 4 were found to be spore forming while 9 were non-spore forming, 3 were found to be motile where as 10 were non-motile.

**Characterization of organism on the basis of Catalase and Oxidase test**

| Sr. No. | Sample Code | Catalase test | Oxidase test |
|---|---|---|---|
| 1 | 3a | - | + |
| 2 | 4a | - | + |
| 3 | 5a | - | - |
| 4 | 9a | - | + |
| 5 | 10a | + | - |
| 6 | 19a | - | + |
| 7 | 20a | + | + |
| 8 | 22a | + | + |
| 9 | 24a | - | + |
| 10 | 9 | + | - |
| 11 | 10 | + | - |
| 12 | 11 | + | + |
| 13 | 12 | + | - |

The isolated organisms can be differentiated on the basis of biochemical test such as Catalase and oxidase. Out of 13 isolates 7 were catalase positive and 6 were catalase negative similarly 8 were oxidase positive and 5 were oxidase negative.

| Sr. No. | Name of Microorganism | Amylase |
|---|---|---|
| 1 | *Enterococcus durans* | - |
| 2 | *Bacillus sp.* | + |
| 3 | *Micrococcus varians* | - |
| 4 | *Bacillus sp.* | + |
| 5 | *Pediococcus halophilus* | - |
| 6 | *Enterococcus faecium* | - |
| 7 | *Alkaligens sp.* | + |
| 8 | *Bacillus sp.* | + |
| 9 | *Bacillus sp.* | + |
| 10 | *Planococcus kocurii* | - |
| 11 | *Planococcus kocurii* | - |
| 12 | *Micrococcus agilis* | - |
| 13 | *Pediococcus halophilus* | - |

These isolates were tested for their enzyme production. From this observation, the Bacilli and cocci which produce enzyme amylase, protease and lipase were separated out. 5 Bacilli were able to produced amylase and all Cocci can unable produced enzyme amylase. Bacilli did not produce enzyme protease on the other hand 5 Cocci can produced enzyme protease. Similarly 3 Bacilli and 6 Cocci produced enzyme Lipase.

**Zone of Hydrolysis of Starch by isolated microorganisms**

| Sr. No. | Organism Name | Amylase | |
|---|---|---|---|
| | | 24 h | 48 h |
| 1 | *Enterococcus durans* | - | - |
| 2 | *Bacillus sp.* | - | 10 |
| 3 | *Micrococcus varians* | - | - |
| 4 | *Bacillus sp.* | - | 12 |
| 5 | *Pediococcus halophilus* | - | - |
| 6 | *Enterococcus faecium* | - | - |
| 7 | *Alkaligens sp.* | - | 14 |
| 8 | *Bacillus sp.* | - | 15 |
| 9 | *Bacillus sp.* | - | 11 |
| 10 | *Planococcus kocurii* | - | - |
| 11 | *Planococcus kocurii* | - | - |
| 12 | *Micrococcus agilis* | - | - |
| 13 | *Pediococcus halophilus* | - | - |

Zone of starch hydrolysis was obtained after 48h and the maximum zone was shown by the *Bacillus sp* (22a) was 12-15mm. With the help of this result, the organism species was chosen for the isolation of enzyme amylase (22a *bacillus sp*.). Amylase is a group of enzymes whose catalytic function is to hydrolyze (breakdown) sugar and starch to give diverse products including dextrin, and progressively smaller polymers composed of glucose units. The a-amylase family comprises a group of enzymes with a variety of different specificities that all act on one type of substrate being glucose residues linked through an alpha-1-1, alpha-1-4, alpha-1-6, glycosidic bonds. The spectrum of amylase application has widened in many other fields, such as clinical, medical, and analytical chemistries, as well as their wide spread application in starch saccharification and in the textile, food, fermentation, and paper and brewing industries. Total 13 isolates were isolated from bat guano of Lonar Lake. Out of which only 5 bacilli were able to produce an enzyme amylase. The production of enzyme activity by these 5 microorganisms was tested by observing zone of hydrolysis of starch agar plates after 48h incubation. So, from the observation of zone of hydrolysis by *Bacillus sp* (22a) culture was further proceeding for production and characterization. After 48h of incubation in broth condition the *Bacillus sp* has yielded enzyme and it has given good results and its optimum activity at pH 9.5 and its activity at 500C which shows its thermostable nature and hence this culture is industrially important.

Production of alkaline amylase was done by using bacteria *Bacillus sp*, which was isolated from bat guano of Lonar Lake. There were total 5 isolates which have ability to produce alkaline amylase enzyme. The organism that is Bacillus sp (22a) was selected to produce alkaline amylase in large amount. The selection of organism for the production of alkaline amylase is depending on the zone of hydrolysis of starch on media C on point inoculation. *Bacillus sp* gave the highest zone of hydrolysis of starch on media C, hence was selected for the production of alkaline amylase for its characterization. *Bacillus sp* (22a) is an organism that was isolated from the bat guano of Lonar Lake at pH 10-11. The colonies obtained were circular, colorless and having convex elevation. The bacteria was Gram positive long rod, arranged singly, motile, nonsporulating. It is catalase and oxidase positive organism and ferments near about all the sugars. Organism is inoculated ion the broth having pH 10 and incubated at 37 $^{0}$C. For 24h, 48h, and 72h, and tested for the characterization by using effect of pH, temperature, enzyme concentration and substrate concentration.

**Morphological and Biochemical tests for *Bacillus sp* (22a)**

| Sr. No. | Test | Observation |
|---|---|---|
| 1 | Isolation Media | C |
| 2 | Shape of Colony | Circular |
| 3 | Color | White |
| 4 | Elevation | Convex |
| 5 | Gram Reaction | + |
| 6 | Spore Reaction | - |
| 7 | Motility | + |
| 8 | Shape | Long Rod |
| 9 | Arrangement | Single |
| 10 | Catalase | + |
| 11 | Oxidase | + |
| 12 | Lactose | + |
| 13 | Dextrose | + |
| 14 | Mannitol | + |
| 15 | Fructose | + |
| 16 | Sucrose | + |

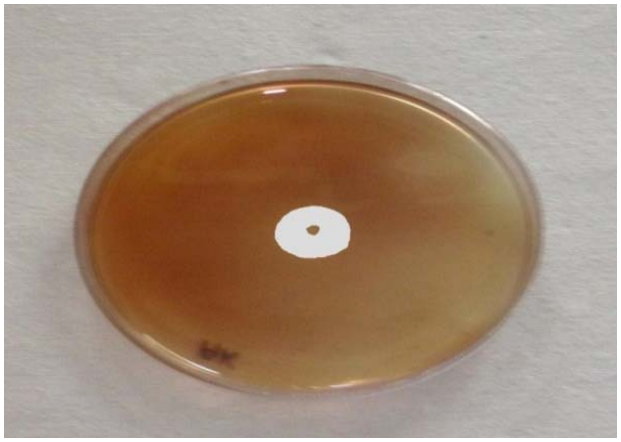
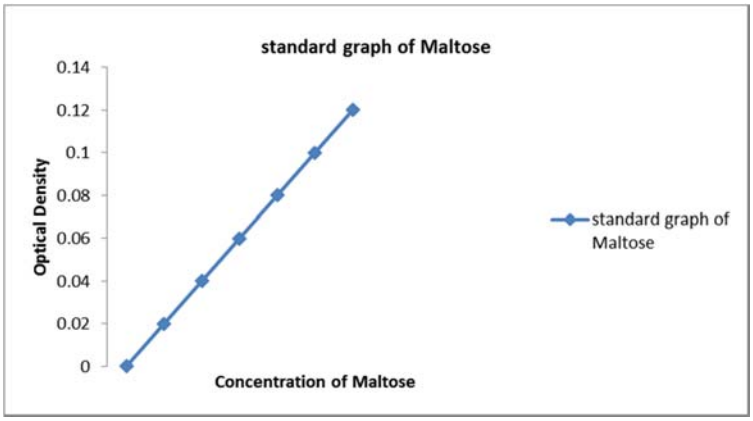


**Fig :** Zone of hydrolysis of Starch by the bacteria isolated from Bat Guano of Lonar Lake.

**Characterisation**

**Optical Density for standard graph of Maltose**

| Test tube No. | Blank | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| O.D. | 0.00 | 0.02 | 0.04 | 0.06 | 0.08 | 0.10 | 0.12 | 0.15 | 0.13 | 0.18 | 0.19 |
| Conc. of Maltose | 0.00 | 0.05 | 0.10 | 0.15 | 0.20 | 0.25 | 0.30 | 0.35 | 0.40 | 0.45 | 0.50 |




**Standard graph of Maltose**

Standard graph of Maltose was obtained by plotting the values of Concentration of Maltose against the Optical Density obtained at 540 nm. The graph was used for the calculation of concentration of Maltose at various parameters**.** At various concentration of Maltose such as 0.05, 0.1, 0.15,etc the optical density was checked and the straight line obtained. various physico-chemical characters such as pH, Temperature, Enzyme Concentration and Substrate Concentration were studied by the help of standard graph.

**Effect of Temperature**

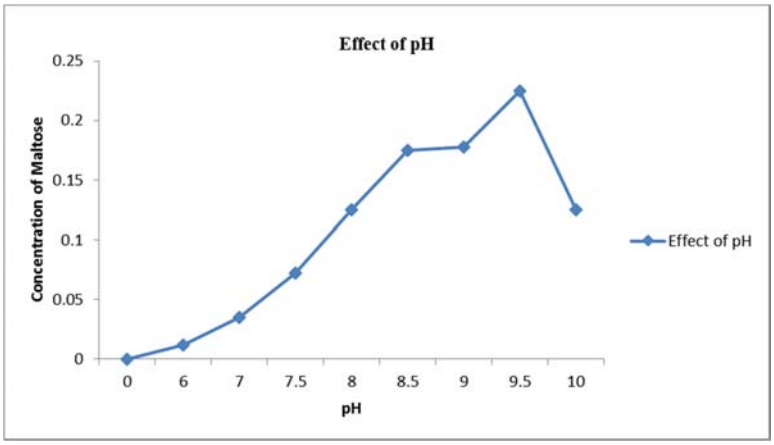
| 1 | Temperature | Blank | Control | $40^0$ C | $50^0$ C | $60^0$ C | $70^0$ C | 80 $^0$C | 90 $^0$C |
|---|---|---|---|---|---|---|---|---|---|
| 2 | O.D. | - | 0.096 | 0.125 | 0.169 | 0.148 | 0.132 | 0.126 | 0.118 |
| 3 | Expt- Control | - | - | 0.029 | 0.073 | 0.052 | 0.046 | 0.030 | 0.022 |
| 4 | Conc. of Maltose | - | - | 0.075 | 0.17 | 0.12 | 0.11 | 0.075 | 0.05 |

**Effect of Temperature on the acyivity of enzyme amylase**

The activity of enzyme amylase isolated from *Bacillus sp* (22a) was measured at various temperature. The enzyme has shown its activity between 40 – 90 $^0$C and the optimum activity showed at 50 $^0$C at the O.D. of 540nm and the decreased enzyme activity was observed after 50 $^o$C. This revealed its thermostable character of bat guano enzyme. The alkaline pH stable enzyme are now-a-days are the key component of the various industries. Behal *et al*, (2006) [1] have found optimum temperature at 40 $^0$C while characterization of of AB 04-alpha amylase. Boyer and Ingle (1972) obtained the optimum temperature at 50 $^0$C which has its own importance in industries.

**Effect of pH**

| 1 | pH | Blank | Control | 6 | 7 | 7.5 | 8 | 8.5 | 9 | 9.5 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 2 | O.D. | - | 0.031 | 0.036 | 0.047 | 0.060 | 0.082 | 0.095 | 0.103 | 0.120 | 0.083 |
| 3 | Expt-Control | - | - | 0.005 | 0.016 | 0.029 | 0.051 | 0.064 | 0.072 | 0.089 | 0.052 |
| 4 | Conc. of Maltose | - | - | 0.012 | 0.035 | 0.072 | 0.125 | 0.175 | 0.178 | 0.225 | 0.125 |

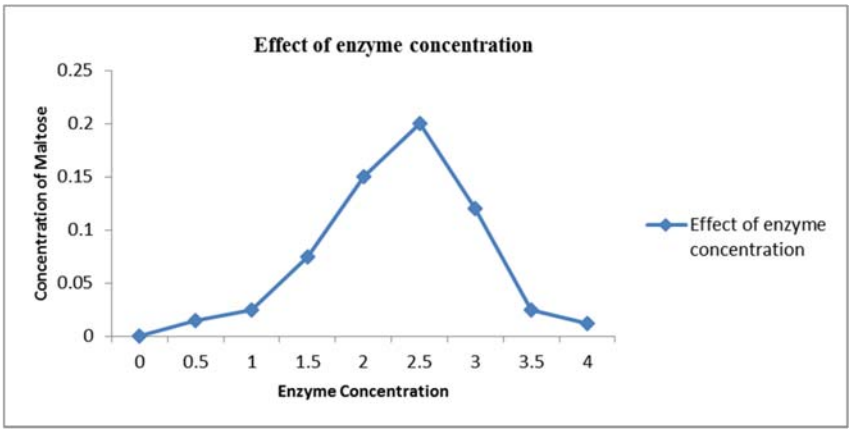


**Effect of pH on activity of enzyme amylase**

The pH was checked for enzyme amylase and the optimum pH for enzyme amylase obtained from bat guano of Lonar Lake was 9.5 from *Bacillus* sp at the O.D. of 540nm. The enzyme showed less activity at the pH of 6 and the activity slowly increased after attaining the pH of 8.0. With the increase in the pH from 6 to 10, the enzyme activity also increased, showing optimum activity at pH 9.5 and after pH 9.5 it declined. Boyer and Ingle (1972) have obtained the pH for selected extracellular alkaline amylase i.e. at pH 9.2. Similarly, Joshi *et al* have observed the pH for selected strains in the range of 9-11 which shows alkaline environment. Similar results were obtained from the present study showing alkalophilic nature of the enzyme amylase.

**Effect of Enzyme concentration**

| 1 | Conc. of Enzyme. | Blank | Control | 0.5 | 1.0 | 1.5 | 2.0 | 2.5 | 3.0 | 3.5 | 4.0 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 2 | O.D. | - | 0.070 | 0.074 | 0.080 | 0.099 | 0.130 | 0.156 | 0.125 | 0.083 | 0.073 |
| 3 | Expt- Control | - | - | 0.004 | 0.010 | 0.029 | 0.060 | 0.086 | 0.055 | 0.013 | 0.003 |
| 4 | Conc. of Maltose | - | - | 0.015 | 0.025 | 0.075 | 0.15 | 0.2 | 0.12 | 0.025 | 0.012 |

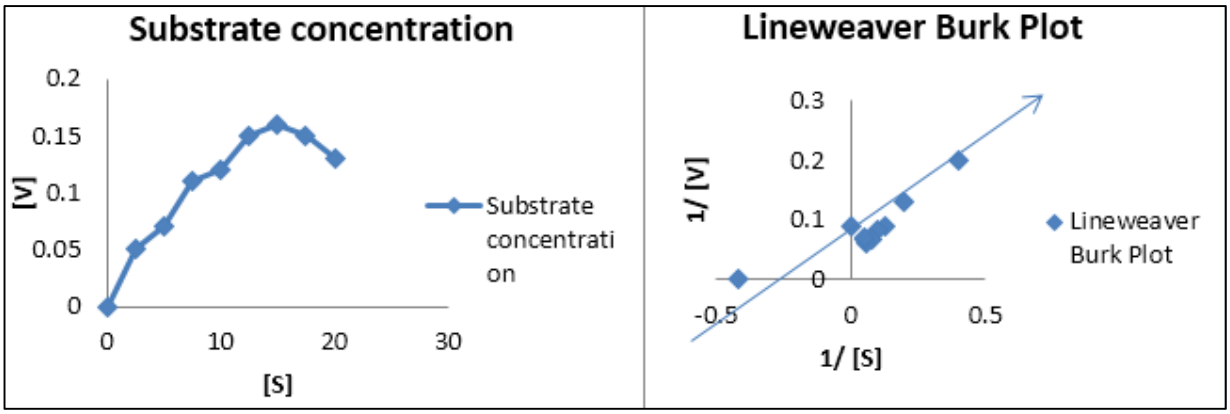



**Effect of enzyme concentration on activity of enzyme amylase**

In certain instances, when all the variables are controlled the enzyme activity is depend upon enzyme concentration. The rate of enzyme action increases with the increase in enzyme concentration upto certain limit. But the further increase in the enzyme concentration decreasesm the rate of enzyme activity. At various concentrations such as 0.05, 0.1, 0.15, 0.2 and 0.25mg of substrate an enzyme has shown variable level of activity. It has shown its activity from 0.015 to 0.025 giving its maximum enzyme activity at 0.2mg/mL of substrate and enzyme amylase obtained from bat guano of Lonar Lake has shown its optimum activity at 2.5 mg/mL of enzyme concentration at the O. D. of 540nm and at this concentration enzyme worked optimally so as to catalyse its substrate.

**Effect of Substrate Concentration**

| Sr. No. | O.D. | V | 1/V | S | 1/S |
|---|---|---|---|---|---|
| Control | 0.031 | - | - | - | - |
| 1 | 0.052 | 0.05 | 20 | 2.5 | 0.40 |
| 2 | 0.061 | 0.07 | 13 | 5.0 | 0.20 |
| 3 | 0.075 | 0.11 | 9.09 | 7.5 | 0.13 |
| 4 | 0.081 | 0.12 | 8.33 | 10.0 | 0.10 |
| 5 | 0.085 | 0.15 | 6.66 | 12.5 | 0.08 |
| 6 | 0.086 | 0.16 | 6.25 | 15.0 | 0.06 |
| 7 | 0.085 | 0.15 | 6.66 | 17.5 | 0.05 |
| 8 | 0.083 | 0.13 | 7.69 | 20.0 | 0.05 |




**Effect of substrate concentration on activity of enzyme protease**

The selected organism was tested for the effect of substrate cincentration on the activity of enzyme amylase. The Michaelis-Menten graph showed the two important values i.e. Km and Vmax. The smaller Km value indicates the higher enzyme affinity towards the substrate which show the substrate affinity of enzyme. By plotting Michaelis-Menten

graph the optimum Km obtained for amylase enzyme was 2.25mg/mL. At this substrate concentration enzyme showed its optimum activity and its substrate concentration by Line-WeaverBurkplotwas2.38mg/mLattheO.D.of540nm.

**Conclusion**

It is concluded that out of thirteen isolates, five isolates have shown the production of alkaline amylase. *Bacillus sp* 22a showed highest zone of starch hydrolysis (15mm). Enzyme amylase obtained from the *Bacillus sp* 22a isolated from bat guano of Lonar Lake, has its optimum temperature activity at 50 $^{0}$C and having optimum pH at 9.5.Similarly, enzyme concentration has shown its optimum enzyme activity at 0.2 mg/mL and its substrate concentration at 2.25 mg/mL by Michaelis-Menten plot and by Line-weaver Burk plot showed 2.38 mg/mL. By performing the study of various parameters, it was concluded that the enzyme amylase obtained from *bacillus sp* 22a isolated from bat guano of Lonar lake has its own importance by the industrial point of view and may be used in various industries such as paper, cloth, detergent, etc.

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