Effect of different parameters on the production of alkaline α-amylase from *Bacillus subtilis*

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Abstract:

Alkaline α –amylases are used in the starch, textile industries and as an ingredient in detergents. Water samples are collected from Lonar lake of Buldana district (M.S.). The collected water samples are analyzed for isolation of bacteria on the nutrient agar which directly prepared in Lonar lake water. Bacterial isolate was identified on the basis of cultural, morphological and biochemical characterization. The results are found that the *Bacillus subtilis* produced efficient zone of starch hydrolysis on the starch agar by producing the enzyme alkaline α - amylase. The optimum α - amylase production was studied by using different carbon and nitrogen sources, different pH, incubation temperature and time (hrs). Then assay of the activity of alkaline α amylase performed by DNSA method. The optimum alkaline amylase production was observed in the 1% dextrose and starch as a carbon source and 0.5% peptone and 0.5% meat extracts as a nitrogen source, pH 10.00, incubation temperature 40^{0}C and incubation time 72 hrs.

Keywords:

Bacillus subtilis, incubation temperature and alkaline α – amylase.

Introduction:

Thousands of enzymes are found in living cells where they act as catalysts for the thousands of biochemical reactions which occur. In addition to making life possible, many enzymes have numerous applications that affect our daily lives in other ways such as food processing, clinical diagnoses, sewage treatment, and the textile industry (Miller, 1992; Waghode and Garode, 2013). Alkaline amylases that have optimum pH values higher than 8.0 have potential applications for hydrolyzing Starch under high pH conditions used in the Starch and textile industries and as an ingredient in detergents for automatic dishwashers and laundries.(Ozaki and Tanaka, 1990).

Amylases having approximately 25% of the enzyme market have almost completely replaced chemical hydrolysis of Starch in Starch processing industry (Pandey*et al.,* 2000). Alkaline α-amylases can be useful in related applications and retain activity at the pH at which detergents function. This work reports the influence of media composition on alkaline amylase production from *Bacillus subtilis*CB-18 isolated from the soil samples (Ogbonnaya and Odiase, 2012).

The attempt was made to study the effect of different Carbon and Nitrogen sources, different pH, incubation temperature and time (hrs) on the activity of alkaline α-amylase from source *Bacillus subtilis.*

Material and Methods:

Water samples are collected from Lonar crater and analyzed for isolation of bacteria. Bacteria are isolated on the nutrient agar which directly prepared in Lonar lake water. The standard Hi- Medias were used for the work. The bacterial isolate screened for α-amylase activity by amylase assay on starch agar with pH 10.5. The isolate of bacteria is characterized and identified according to Bergey's manual of determinative bacteriology (Holt, *et al.*, 1994; Olajuyigbe*et al.*, 2005).

Effect of different parameters on alkaline α –amylase production:

The different parameters were used for the production ofalkaline α – amylase from *Bacillus subtilis.* The different carbon sources of growth were used in 1% concentration such as Starch, Maltose, Lactose, Dextrose, Sucrose, and Mannitol and nitrogen sources were used in 0.5% concentration as inorganic nitrogen sources such as Ammonium chloride, Ammonium sulfate and organic nitrogen sources such as Peptone, Tryptone, Meat extract and Yeast extract. The different pH such as (8.00, 9.00, 10.00 and 11.00), different incubation temperatures such as (20, 30, 40 and 50^{0}C) and different incubation times such as (24, 48, 72 and 96 hrs) were used for the production of alkaline α- amylase. By using different parameters, the optimum productions of alkaline α- amylase production were studied from *Bacillus subtilis*. The isolate is grown in basal media on laboratory scale and cells are removed by centrifugation, the supernatant

is used as crude enzyme preparation (McTigue*et al.*, 1995). The routine enzyme assay is used for alkaline amylase activity involved measuring the reducing sugars resulting from the hydrolysis of soluble starch. The Di-Nitro-Salicyclic acid (DNSA) reagent method is used for assay (Miller, 1959).

Result and Discussion:

Production of alkaline α - amylase enzyme from *Bacillus subtilis*was done by using the different parameters such as pH, temperature, incubation time, Carbon and Nitrogen sources. The different pH such as 8.0, 9.0, 10.0 and 11.0 were used for the enzyme production. The optimum pH was found 10.0 where α - amylase enzyme formation was 4.85 mg. The different temperature such as 20, 30, 40 and 50^{0}C were used for the enzyme production. The optimum enzyme production was seen at temperature 40^{0}C where α - amylase formation was 4.75 mg. The effect of different incubation time such as 42, 48, 72 and 96 hrs was used for α - amylase enzyme production. The optimum enzyme production was seen at 72 hrs incubation where α - amylase formation was 4.95 mg which was shown in figure 1.

The different six type of Carbon sources such as Dextrose, Lactose, Mannitol, Sucrose, Starch and Maltose were used for the production of alkaline α - amylase enzyme. In the 0.5%, 1.0% and 1.5% of Dextrose were shown maximum velocity of α - amylase enzyme formation as 4.85, 5.30 and 5.60 mg respectively which were shown in figure 2. The different six types of Nitrogen sources such as organic Nitrogen sources Yeast Extract, Meat Extract, Tryptone, Peptone and inorganic Nitrogen sources Ammonium Chloride and Ammonium Sulphate were used for the production of enzyme. These Nitrogen sources were used in different concentration such as 0.5 and 1.0% and 1.0% Starch was used as control. Carbon source for the production of enzyme which was shown in figure 3. Maximum velocity of α - amylase enzyme production was seen at 0.5% Peptone which was 5.10 mg.

The maxiumim production of alkaline α-amylase was seen in the 1% Dextrose and Starch with different Carbon sources and 0.5 % Peptone as a Nitrogen source which were used for optimization of enzyme production from the *Bacillus subtilis*. The optimum production of alkaline α - amylase was seen at pH 10.0, 40^{0}C and 72 hrs incubation time, in the 1% Dextrose and Starch as a Carbon source and 0.5% Peptone Nitrogen source.

Vijayalakshmi*et al.*, in (2012) were found that the maximum enzyme production was found after 48 h of incubation at temperature 40^{0}C and pH 7.00. The optimal temperature and pH for enzyme amylase activity from *Bacillus subtilis* KC3 were 50^{0}C and 6.5 respectively.

Yadav*et al.*, in (2012) found that the nature and amount of Carbon and Nitrogen sources in culture media are also important for the growth and production of extracellular amylase in bacteria along with the physical parameters. The levels of amylase in the crude culture supernatants varied greatly in response to the Carbon and Nitrogen source used for the growth of the *Bacillussubtilis*strain. Peptone and Starch in media were promoted α-amylase productivity.

Gomaa in (2013) reported that supplementation of Carbon sources in the form of monosaccharide, disaccharides and polysaccharides resulted in marginal increase in α-amylase production by *Bacillus subtilis*.

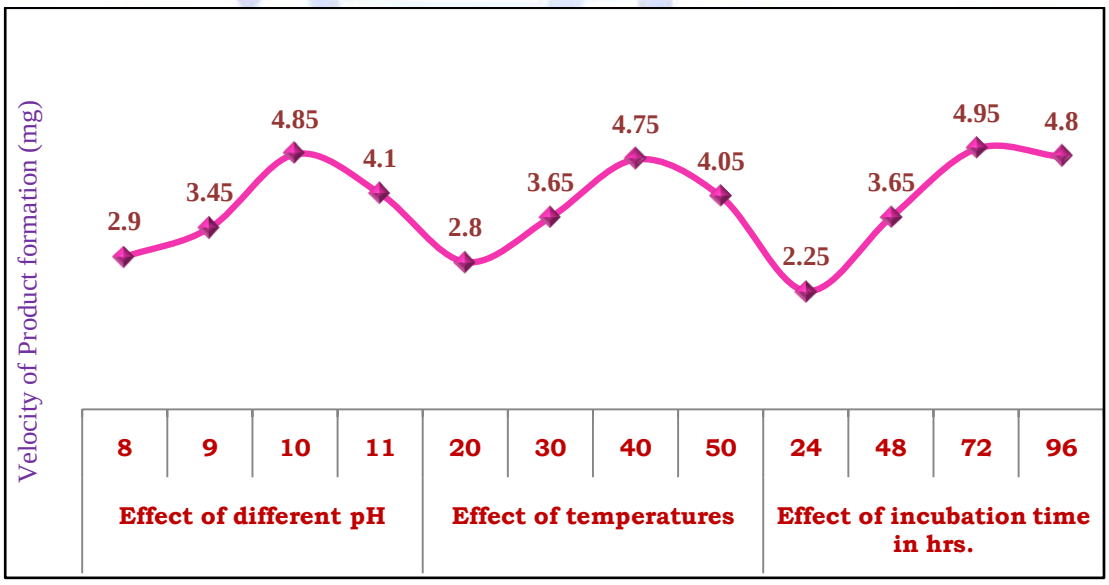

Figure. 1 Effect of diferent parameters on enzyme α- amylase Production from *Bacillus subtilis*

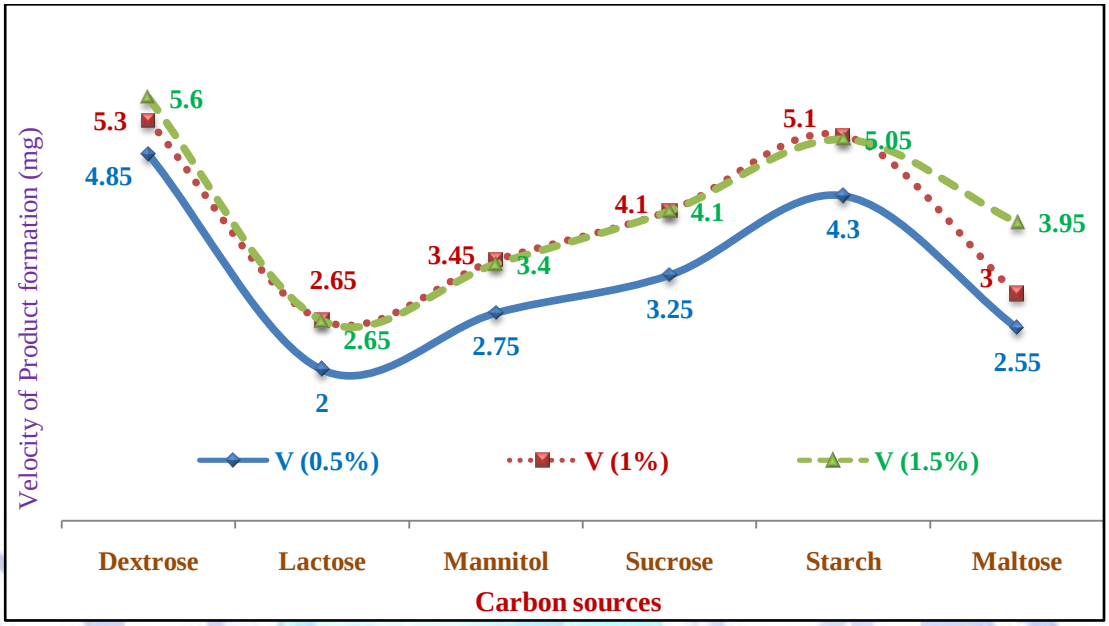

Figure. 2 Effect of Carbon sources on enzyme α- amylase production from *Bacillus subtilis*

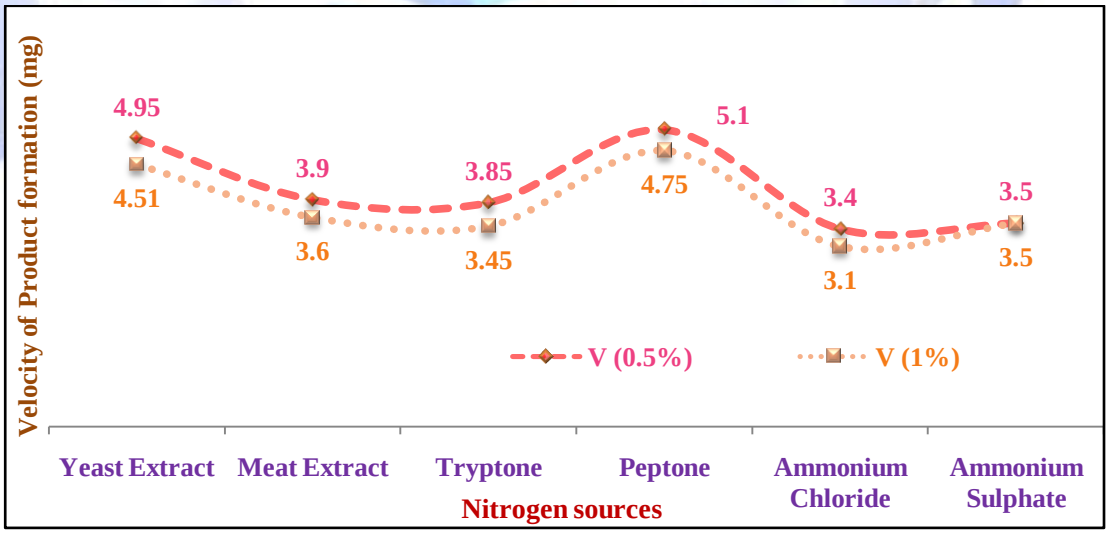

Figure. 3 Effect of Nitrogen sources on enzyme α- amylase production from *Bacillus subtilis*

Conclusion:

Alkaline amylases are used in the starch, textile industries and as an ingredient in detergents. *Bacillus subtilis*is a potential producer of

extracellular α-amylase which could find applications in industry and biotechnology. The results obtained in this study show that there is appreciable high production of enzyme alkaline α-amylases. The optimum alkaline amylase production was observed in the 1% dextrose and starch as a carbon source and 0.5% peptone and 0.5% meat extracts as a nitrogen source, pH 10.00, incubation temperature 40^{0}C and incubation time 72 hours. The culture conditions and media components were optimized for better production of both the enzymes. The enzyme thus is produced presently under optimization.

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