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# Production, Optimization and Characterization of Alpha Amylase Isolated from Wastewater

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## ABSTRACT

The study produced, optimized and characterized α-amylase from a bacterium isolated from waste water with a view to obtaining best optimized conditions required for growth of the organism for the production of the enzyme for industrial uses. The waste water collected from Daula restaurant located in Birnin Kebbi were taken to laboratory and analysed. Isolates from the plates were screened for amylase activity using starch agar and are detected on potato starch solution. The bacterium with the highest amylase activity was selected for enzyme production. Optimal conditions for enzyme production by the bacterium were determined. The best isolate from the waste that showed better ability for amylase production was identified molecularly as *Lysinibacillus sphaericus* C4-31. The peak amylase activity was observed at day 4 of incubation (3.44mM/min). The optimum pH and temperature for the production of *Lysinibacillus sphaericus* C4-31 α-amylase was 8 and 30°C respectively. The result also revealed that ammonium phosphate supported higher enzyme activity of 5.86mM/min among the nitrogen source. Glucose as a carbon source gave the highest activity of 6.89mM/min. The study concluded that α-amylase can be synthesize by *Lysinibacillus sphaericus* C4-31which is moderately thermostable and able to degrade many cheap raw starches and can therefore find applications in the food industry.

**Keywords:** Enzymes; restaurant waste; bacteria; amylase.

## INTRODUCTION

Microbial enzymes have a great number of applications in food, pharmaceutical, textile, paper, leather and other industries (Hasan *et al*., 2006). Their applications have been increasing rapidly. Among industrially important enzymes, hydrolases come in the first place and include enzymes with a wide substrate specificity. Carbohydrases, proteases, pectinases and lipases are classified into hydrolases. They catalyze the hydrolysis of natural organic compounds (Sundarram and Krishna, 2020; Underkofler *et al*., 2007; Rajan, 2001). A special focus on amylase from a warm-adapted bacterium in this particular study. The α-amylases are enzymes that hydrolyze starch molecules to generate progressively smaller polymers composed of glucose units (Windish *et al.*, 2005). The enzyme accounts for 65% of enzyme market in the world. To meet the growing demands in the industry, it is necessary to improve the performance of the system and thus increase the yield without increasing the cost of production (Gangadharan *et al*., 2008).

Today, a large number of microbial amylases have almost completely replaced the chemical hydrolysis of starch. The main advantage of using microorganisms for the production of amylase is the ability to bulk produces the enzyme and the easy manipulation of microbes to achieve enzymes with desired characteristics. Moreover, the stability of microbial amylases are higher than those from plant and animal sources (Tanyildizi *et al.*, 2005).

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While studies exist on enzyme production from various sources and optimization of parameters like pH, temperature, and carbon/nitrogen sources, a lack of focus on wastewater-derived amylase, especially in the context of industrial-scale applications, presents a key area for further investigation. Therefore there is need in translating findings from laboratory-scale amylase production to real-world industrial applications. This study focused on demonstrating the characterization of wastewater-derived amylase and the type of microbe that could tolerate, and produce amylase so a sto be used in specific industrial processes.

## METHODOLOGY

### Microbial and innoculum preparation

*Lysinibacillus sphaericus* C3-41 was isolated from dietary oil rich waste water as characterized and identified molecularly. The isolate was and re-cultured at laboratory of the Department of Microbiology, Landmark University, Omu-Aran, Kwara State, Nigeria for further analysis. *Lysinibacillus sphaericus* C3-41 cell free supernatant was obtained by growing the organism in a sterile PBS buffer, pH 7.2 and centrifuging the broth culture. One hundred (100 µl) of the sixth 10-fold serial dilutions were plated on De Man Rogosa and Sharpe (MRS) agar, followed by picking some colonies at random and growing in MRS broth at $30^0$C for 24hrs (Sundarram and Krishna, 2020). The broth culture was then centrifuged at 10,000 rpm for 5mins. Cell pellets harvested from the MRS broth cultures were re-suspended in MRS broth containing 15% glycerol and aliquots were frozen for use when needed. Cell free supernatant was used as the crude enzyme for assay and further analysis (Das *et al*., 2004).

### Preparation of Wastewater and medium for Enzyme production

Wastewater treatment was done by distributing sterile wastewater into five portions of 100 mL each contained in 250 mL Erlenmeyer flasks followed by inoculating 1 mL *Lysinibacillus sphaericus* C3-41 culture (1.86 x $10^6$ CFU/ml) into the wastewater sample contained in each flask and the absorbance was measured at 600nm (Gupta *et al.*, 2004). The flasks were kept in shaking incubator with 150 r.p.m at 37°C. Samples were drawn from each of the flasks at intervals of 6 h for a period of 24 h and later centrifuged at 5000 x g for 1 minute at 4°C. The medium for amylase production contains (g/L): soluble starch (10), peptone (20), $MgSO_4.7H_2O$ (1.0), $Na_2HPO_4$ (3), $FeSO_4$ (0.3) and NaCl (0.1), which was sterilised then left to cool until 27 °C. One mL of 24 h culture of *Lysinibacillus sphaericus* C3-41 (0.5 McFarland) was inoculated to 99 mL of cultivation medium containing (soluble starch (10), $(NH_4)_2SO_4$ (2), $MgSO_4.7H_2O$ (1.0), $Na_2HPO_4$ (3), $FeSO_4$ (0.3) and NaCl (0.1)) in a 250 mL volumetric flask and allowed to incubate for 48 h at 45 °C with agitation (150 rpm) (Msarah *et al*., 2020). Culture samples (1mL) were removed at 6h intervals and centrifuged at 10,000g for 10min. Growth was measured using turbidity of harvested samples at 600 nm. The supernatant obtained after centrifugation was used as crude enzyme solution. The enzyme was usually stored at 4°C until when needed.

### Assay for Amylase Activity of *Lysinibacillus sphaericus* C3-41

In the assay for Amylase activity, 0.5 mL of the supernatant was added into a tube containing 1.5 mL of 2 % (w/v) of potato starch solution and 1 mL of 0.05 M acetate buffer, pH 5.0. The reaction mixture was incubated at $40^0$C for 15 min in water bath. Then, 1 mL of the mixture was transferred to a new tube containing 1 mL of 3, 5-dinitrosalicylic acid (DNS) reagent and kept in boiled water for 10 min (Mawadza *et al.*, 2000). The colour density was determined spectrophotometrically at 540 nm. One international unit (IU) of amylase activity was considered as the required amount of the enzyme to release 1 µmol of reducing sugar from the sugar source within one minute under standard experimental conditions (Rao *et al*., 2011).

### Purification of amylase enzyme

Purification of amylase enzyme was achieved by ammonium sulphate precipitation followed by dialysis. One hundred milli litre (100 ml) of cell-free extract was saturated with centrifuged at 7000 rpm for 15 min. The supernatant was collected and saturated up to 0–30 and 30–80% with ammonium sulphate. Then, the content was centrifuged at 7000 rpm for 15 min and the pellet was collected for further analysis (Rao *et al*., 2011). The enzyme mixture was transferred in a dialysis bag with space size 70 cm and immersed in phosphate buffer pH

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7 at 4°C for 24 hr. The buffer was continuously stirred using a magnetic stirrer throughout the process. The buffer was changed several times during the process in order to obtain proper purification.

**Optimization of conditions for Microbial growth and Amylase production**

Various process parameters affecting enzyme production were optimized. Such different growth conditions were optimized independent of each other. The parameters investigated included (i) incubation time (24h-12days), (ii) incubation temperature (20-80°C), (iii) pH of medium (4-8), (iv) nitrogen source (ammonium nitrate, ammonium nitrite, ammonium sulphate, ammonium chloride, ammonium carbonate and ammonium phosphate; 1%w/v), (v) supplementary carbon source (glucose, fructose, galactose, sucrose, maltose, lactose; 1%w/v), and (vi) salt ions (Na, K, Mg, Ca, Fe $PO_4$, $SO_4$, $Cl^-$). Inoculation of the organism into media without supplement serves as control (Gupta *et al.,* 2004).

**Growth and amylase production of *Lysinibacillus sphaericus* C3-41at different incubation periods**

Amylase production was carried out for 12 days and the samples were collected after every 24hours to amylase production and microbial growth. Enzyme assay was carried out using standard assay procedure as described by Mawadza *et al.* (2000).

**Effect of temperature on growth and amylase production by *Lysinibacillus sphaericus*C3-41**

The optimum temperature for amylase activity was determined by incubating the assay mixture described above at different temperatures between 20°C and 80°C (at 10°C intervals) for 24hours while keeping other parameters constant and the resulting enzyme activity using standard assay procedure was determined as described by Mawadza *et al.* (2000).

**Effect of pH on growth and amylase production by *Lysinibacillus sphaericus*C3-41**

Enzyme production and the growth of *Lysinibacillus* sp were observed on the media with varying pH ranging between pH 4 to pH 8, while keeping other parameters constant and the resulting enzyme activity using standard assay procedure was determined as described by Mawadza *et al.* (2000).

**Effect of various nitrogen sources on growth and amylase production by *Lysinibacillus sphaericus*C3-41**

The influence of ammonium nitrate, ammonium nitrite, ammonium sulphate, ammonium chloride, ammonium carbonate and ammonium phosphate (1%w/v) each was examined on the growth and amylase production of *Lysinibacillus* sp. in the basal medium while keeping other parameters constant. Enzyme assay was carried out using standard assay procedure (Francisco *et al.,* 2004).

**Effect of various carbon sources on growth and amylase production**

Starch present as carbon source in the production medium was replaced with different carbon sources like glucose, fructose, galactose, sucrose, maltose and lactose at 1% v/v. The control contains the media without carbon supplement. Enzyme assay was carried out using standard assay procedure.

**Effect of Metal and non-metals on the growth and amylase production**

Effect of metals and non metals were examined on the growth of the organism. Metals such as sodium (Na), potassium (K), magnesium (Mg), calcium (Ca), iron (Fe), and non-metals such as phosphate ($PO_4$), sulphate ($SO_4$) and chloride (Cl) each at (1mg/100 vol of water) were used. They resulting enzyme activity was determined using spectrophotometry as described by Mawadza *et al.* (2000) using standard assay procedure

## RESULTS AND DISCUSSION

Many amylase producing organisms were reported previously from the source of soil marine (Mohsen *et al*., 2018). Cost of fermentation medium is one of the important factors in microbial enzyme production and

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utilization of several waste sources can play a vital role in the trimming down these costs. Microorganisms isolated from local wastes, when combined with cheap substrates can be used to reduce amylase production cost.

**Growth and α-amylase production by *Lysinibacillus sphaericus* at different incubation periods**

Findings from this study showed that optimal amylase production occurred on day 4 with a value of 3.44mM/min while the optimal growth was observed on day 5 of incubation for the organism, following which a decline in growth and amylase production was observed (Figure 1). This results closely agrees with Vishnu *et al*. (2014) who reported that *Bacillus sphaericus* had optimal amylase production after 72h of incubation. This suggests that amylase production was growth dependent. This contrasts with findings by Simair *et al*. (2017) who reported optimal amylase production at 36h during optimization. Qureshi *et al*. (2013) suggested that further decline could be due to decrease in cell growth, a deficiency of nutrients, and a change in the final pH.

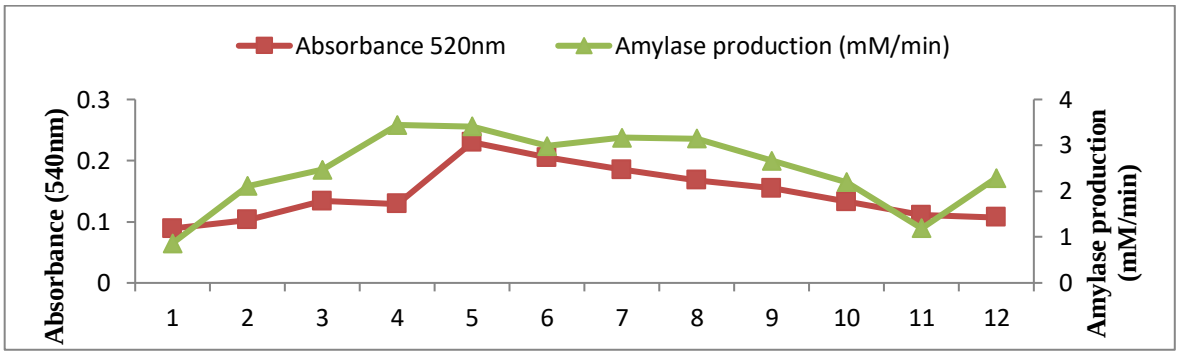


Figure 1: Growth of *Lysinibacillus sphaericus* and amylase enzyme productionat different incubation periods.

**Effect of temperature on α-amylase production by *L. sphaericus***

The effect of temperature on α-amylase production by *L. sphaericus* C3-41 in the sample revealed that enzyme activity peaked at 30°C (Figure 2). The enzyme activity decreased beyond this temperature till 70°C and later dropped sharply at 80°C. The organism grew in the medium at the rate of 0.02 mg/day while it produced the amylase enzyme at the rate of 0.29 mM/min every day. This closely agrees with findings by Akzan *et al*. (2011) who reported maximum amylase production from *Bacillus licheniformis* at 37 °C, further increase in the temperature yielded reduced amylase activity, this could be due to the decreasing microbial growth and denaturation of the enzyme at higher temperatures.

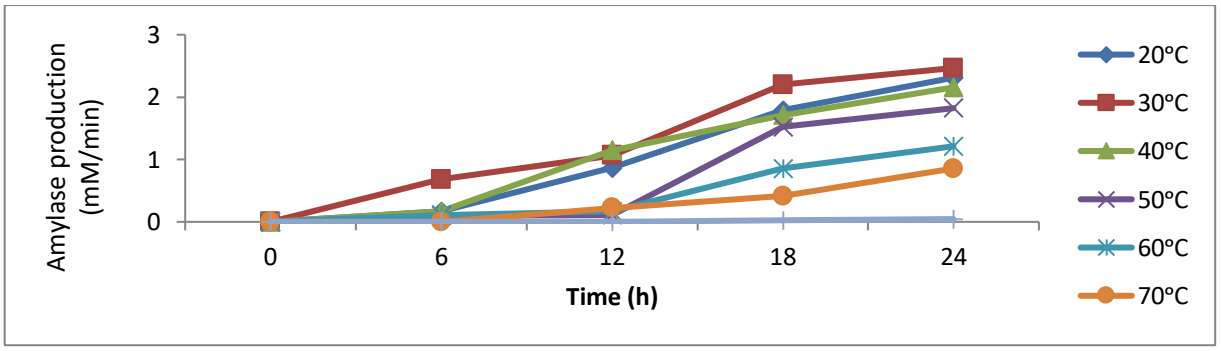


Figure 2: Effect of different temperatures on amylase enzyme production by *Lysinibacillus sphaericus* C3-41

**Effect of pH on α-amylase production by *L. sphaericus*** C3-41

Findings also showed that optimal amylase production occurred at pH 8. Beyond this optimal level, lower pH also resulted in reduction in α-amylase production such that at pH 8, the enzyme produced was 2.89mM/min while pH 7 it produced 2.11mM/min (Figure 3). This finding agrees with Simair *et al.* (2017) who reported optimal amylase production by Thermophilic *Bacillus* sp. BCC 021-50at pH 8. The organism grew in the

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medium at the rate of 0.06 mg/day while it produces the amylase enzyme at the rate of 0.78mM/min every day. Microbial stains possessing thermo-alkaliphilic features offer promises as industrially-important strains owing to their commercial potential, especially alkaline pH-stable amylase, which could be used in detergent formulations.

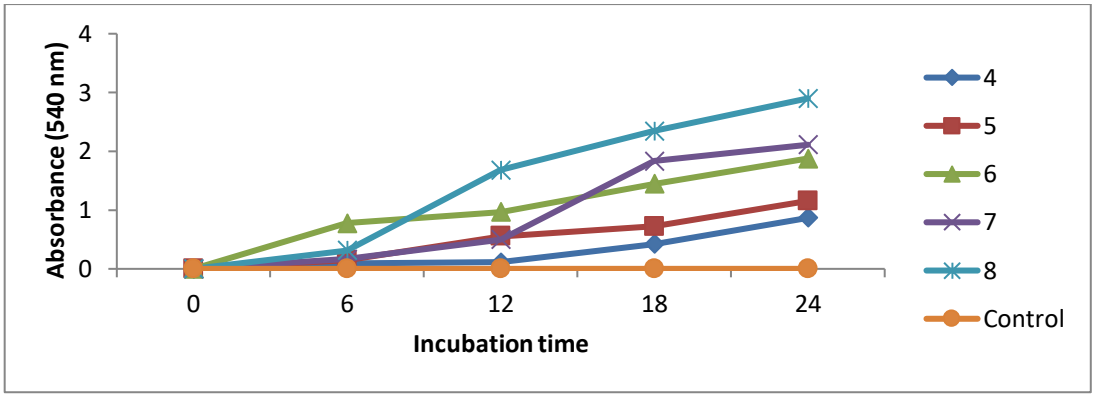


Figure 3: Effect of different pHs on amylase enzyme production by *Lysinibacillus sphaericus*

**Growth and production of amylase by *L. sphaericus*C3-41 in different Nitrogen sources**

Growth and production of amylase by *L. sphaericus* C3-41 in media supplemented with different nitrogen sources showed that ammonium phosphate was a better nitrogen source for *L. sphaericus* C3-41(5.86mM/min) and optimal growth of the organism with 0.281mg. On the other hand, ammonium carbonate was the least favoured nitrogen sources that supported the growth and synthesis of α-amylase by *L. sphaericus* C3-41 (Figure 4).

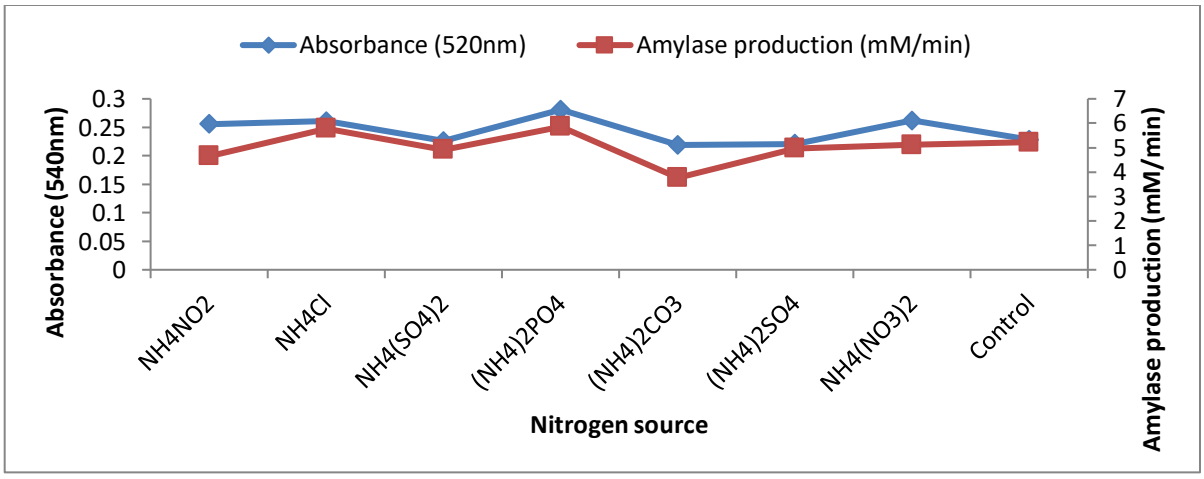


Figure 4: Growth of *Lysinibacillus sphaericus* and amylase production with different nitrogen sources

**Growth and production of amylase by *L. sphaericus*C3-41 in different carbon sources**

Supplementation of carbon sources in the form of monosaccharides, disaccharides and polysaccharides resulted in marginal increase in growth and α-amylase production by *L. sphaericus* C3-41 in domestic waste water. The highest amylase production was observed in medium supplemented with glucose with optimal growth of 0.326mg and amylase production of 6.89mM/min (Figure 5). Higher growth of *L. sphaericus* resulted in increased amylase activity. This is in contrast with the work of Gurudeeban *et al*. (2011) and Sivakumar *et al*. (2011) who reported maltose as best carbon source for amylase production from *Bacillus* sp. Higher glucose production could be due to short incubation time and simple transport mechanism (Hassan and Abd, 2019).. Haq (2003) stated that maltose synthesis may undergo lengthy period due to their characteristic as disaccharide and various mechanisms of maltose transport system which consist of cytoplasmic membrane proteins, a perisplasmic binding protein and a specific outer-membrane porin.

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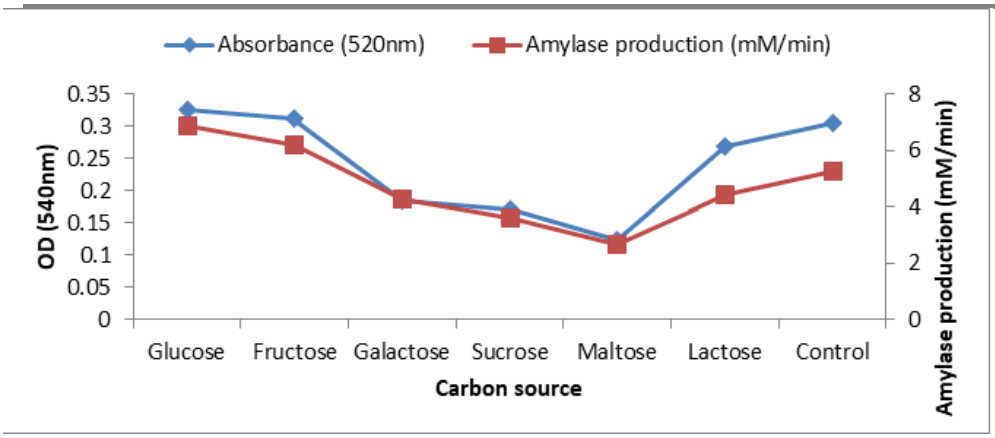


Figure 5: Growth of *Lysinibacillus sphaericus* and amylase production with different carbon sources.

**Effect of Metals and non-metals on the growth of *Lysinibacillus* sp** C3-41 **and amylase production**

Among the different metals and non-metals, the assay mixture supplemented with sodium yielded optimal amylase production. Amylase secretion by the organism also corresponded significantly to the growth of the organism. The maximum amylase produced was 6.55mM/min at a growth of 0.292 mg/L. Reduced growth of *Lysinibacillus sphaericus* and amylase production was observed in the medium supplemented with iron. Also, the sulphate supplemented medium gave the maximum secretion of the enzyme among the non-metallic ions used (Figure 6). This finding differs from reports by Sudha *et al*. (2012) who reported higher amylase production by *Bacillus amyloliquefaciens* $Ca^{2+}$ (0.439) IU/ml/min at 7g/l concentration in comparison to other metal ions. This result shows that metal ions may stimulate the enzyme activity by acting as a binding link between enzyme and substrate combining with both and so holding the substrate and the active site of the enzyme.

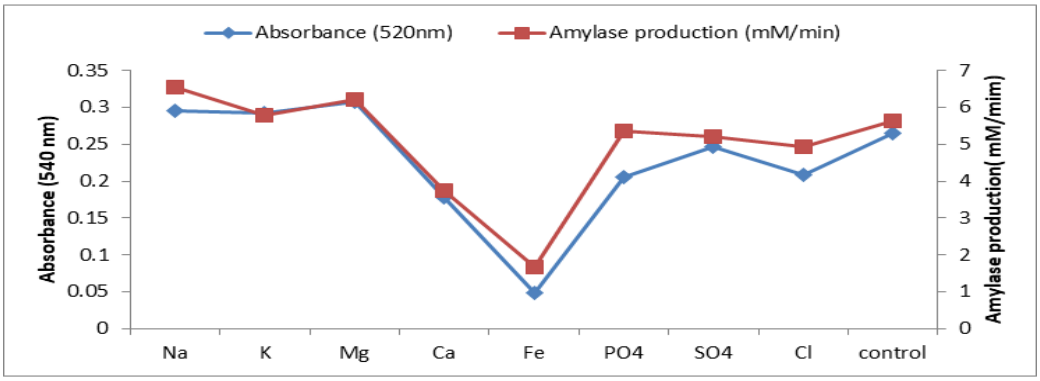


Figure 6: Effect of various metals and non metals on Amylase enzyme production by *L. sphaericus* C3-41

## CONCLUSIONS

This work have revealed that the use of liquid state fermentation for production of α-amylase by *Lysinibacillus sphaericus* C3-41using wastewater as a substrate is an economical process and is very simple to apply. Optimization of the fermentation parameters and the use of suitable carbon and nitrogen supplements resulted in 3 folds increase in the enzyme yield. The enzyme was significantly active at room temperatures and the optimum temperature for the activity was found to be 30°C. The enzyme was found to be active over a wide range of pH and showed the optimum activity at pH 8. These isolates can thus be industrially exploited for the synthesis of α- amylase which can have several industrial applications.

## REFERENCES

1. Baysal Z, Uyar F, Aytekin C (2003). Solid state fermentation for Production of α- amylase by a thermotolerant*B. subtilis*from hot-spring water. Process Biochem. 38:1665-1668.

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2. BhaskaraRao KV, Ashwini K, Gaurav K, Karthik L (2011). Optimization, production and partial purification of extracellular α-amylase from *Bacillus* sp. *marini*. Arch. Appl. Science Res. 3(1):33-42.
3. Das K, Doley R, Mukherjee AK (2004). Purification and biochemical characterization of thermostable, alkaliphilic, extracellular α-amylase from *B. subtilis*DM-03, a strain isolated from the traditional food of India. Biotechnol. Appl. Biochem. 40:291-298.
4. Francis F, Sabu A, Nampoothiri KM, Ramachandran S, Ghosh S, Szakacs G, Pandey A (2003). Use of response surface methodology for optimizing process parameters for the production of α-amylase by *Aspergillusoryzae*.Biochem. Eng. J. 15:107-115.
5. Franscisco, J., Ustariz, L.A., Luis, A.G., and Diaz, M. (2004).Fermentation of individual proteins for protease production by Serratiamarcescens.Biochem*Engineering Journal*, 19: 147-153.
6. Gangadharan D, Sivaramakrishnan S, Nampoothiri KM, Sukumaran RK, Pandey A (2008). Response surface methodology for the optimization of alpha amylase production by *Bacillus amyloliquefaciens*.Bioresour. Technol. 99: 4597-4602.
7. Gangadharan, D., Sivaramakrishnan, S., Nampoothiri, K.M, and Pandey, A. (2008).Solid culturing of *Bacillus amyloliquifaciens*for amylase production.*Food Technology Biotechnology*, 44:269-274.
8. Gupta, R., Gupta, N. and Rathi, P. (2004). Bacterial lipases: an overviewof production, purification and biotechnological properties. *Application MicrobiolBiotechnol*, 64: 763-781.
9. Gurudeeban, S., Satyavaniand, K. and Ramanathan, T. (2011). Production of extra cellular-amylase using *Bacillus megaterium*isolated from White Mangrove (*Avicennia marina*). *Asian Journal Biotechnology*, 3:310-316.
10. Haq I, Ashraf H, Iqbal J, Qadeer MA (2003). Production of alpha amylase by *Bacillus licheniformis*using an economical of medium.Biores. Technol. 87: 57-61.
11. Hassan H, and Abd K K.(2019). Optimization of alpha amylase production from rice straw using solid-state fermentation of Bacillus subtilis. Int. J. Sc. Environ. Technol: 4 (1), 1-16.
12. Hasan, F., Shah, A. A. and Hameed, A. (2006).Industrial applications of microbiallipases.*Enzyme and Microbial Technology*, 39: 235-251.
13. Mawadza, C., Hatti-Kaul, R., Zvauya, R. and Mattiasson, B. (2000).Purification and characterization of cellulases produced by two *Bacillus* strains.*Journal of Biotechnology*, 83(3): 177-187.
14. Mohsen, H., Ali m., and Uygut,. M. (2018). Optimization of alpha-amylase production by Bacillus amyloliquefaciens grown on orange peels. *Iranian J. Sci. Technol. Transactions A: Science*
15. Panneerselvam T, and Elavarasi S.(2015). Isolation of α-amylase producing Bacillus subtilis from Soil. Int. J. Curr. Microbiol. Appl. Sci: 4 (2), 543-552.
16. Rao JLUM, Sathyanarayana T (2003). Enhanced secertion and low temperature stabilization of a hyperthermostable and Ca2+ dependent α-amylase of *Geobacillusthermoleovorans*by surfactants.Lett. Appl. Microbiol. 36:191-196.
17. Sivakumar, T., Ramasubramanian, V., Shankar, T., Vijayabaskar, P. and Anandapandian, K.T.K. (2011).Screening of keratinolytic bacteria *Bacillus cereus* from the feather dumping soil of sivakasi.*Journal Basic & Applied Biology*, 5: 305-314.
18. Suhaimi SN, Phang LY, Maeda T, Abd-Aziz S, Wakisaka M, Shirai Y, Hassan MA (2012). Bioconversion of glycerol for bioethanol production using isolated *Escherichia coli* SS1. Braz. J. Microbiol. 506-516.
19. Sundarram K, and Krishna T P. (2020). α-Amylase Production and Applications: A Review. J. Appl. Environ. Microbiol.: 2 (4), 166-175.
20. Tanyildizi, M.S., Ozer, D. and Elibol, M. (2005).Optimization of amylase production by Bacillus sp. using response surface methodology. Process Biochemistry, 40, 2291-2296.
21. Underkofler, L.A., Barton, R.R. and Rennert, S.S. (2007). Production of Microbial Enzymes and Their Applications.*Applied Microbiology*, 6(3): 212-221.
22. Vishnu, T.S., Soniyamby, A.R., Praveesh, B.V and Hema, T.A. (2014). Production and optimization of extracellular amylase from soil receiving kitchen waste isolate *Bacillus* sp. VS 04. *World Applied Scientific Journal*, 29(7):961-967.
23. Windish, W.W. and Mhatre, N.S. (2005).Microbial amylases. In: Wu, W., Ed., Advances in Applied Microbiology, Elsevier Inc., Houston, 273-304.