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*Research Article*

# Optimization of amylase and lipase enzymes produced by *Bacillus cereus* and *Bacillus subtilis* isolated from waste dumpsites

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**Abstract**
The enzyme amylase is one of the most important in biotechnology, and lipase operates as a catalytic agent for a broad range of hydrolytic and synthetic activities. This study aimed to assess the optimization of amylase and lipase enzyme produced by microorganisms isolated from selected waste or garbage dumpsite in Akure, Ondo State Nigeria. The isolates were identified using biochemical and cultural characteristics. A total of seven bacterial isolates were identified and quantitative production of amylase and lipase by solid-state fermentation was assessed for each bacterial isolate. The optimization of nutritional and environmental parameters on enzymes produced by the isolated organisms was standardized with respect to incubation time, temperature, pH, and carbon and nitrogen sources. The activity of the enzymes generated was determined by spectrophotometric assay. Of the seven organisms isolated, *Bacillus cereus* LA326 and *Bacillus subtilis* AU021 had the highest amylase and lipase activity and were therefore chosen for further research. The maximum level of amylase and lipase activity attained by *B. subtilis* AU021 was 68.0 mmol/min and 16.3 mmol/min after 18 hours of incubation respectively, while the maximum levels of amylase and lipase activity attained by *B. cereus* LA326 were found to be 76 mmol/min after 12 hours and 16.3mmol/min after 18 hours of incubation, respectively. The study found that *B. cereus* and *B. subtilis* isolated from dumpsites could be efficient producers of biotechnology-relevant enzymes and that environmental conditions could influence their enzyme production.

**Keywords**: Amylase, Lipase, Microbe, Optimization, Waste dumpsites

## INTRODUCTION

In many industrialised and developing nations, solid waste disposal is a pervasive issue in both urban and rural locations (Kumar *et al.*, 2014). Solid wastes are non-fluid/non-flowing materials that have been deemed to be worthless or to have no immediate economic demand at a specific place or source, whether they be leftover raw materials, finished goods, out-of-date goods, containers, or after-use materials that have been thrown away (Dutta *et al.*, 2016). Due to the fact that microorganisms are capable of decomposing a variety of pollutants, they have been created for use in the recycling of both toxic and residential solid waste (Srinivasulu *et al.*, 2022).

Microorganisms play a crucial part in recycling carbon and other elements on a global scale, which has been recognised for a long time (Contesini *et al.*, 2018). The various metabolic activities of microbes and their ability to interact with complicated organic and inorganic sub-

strates are currently being utilised to treat solid waste. Through the release of chemical compounds in excess of their threshold limit, urban solid wastes are sources of environmental contamination (Sharma *et al.*, 2022).

Humans can employ enzymes produced by microorganisms for a variety of applications. Lower production costs, the ability to synthesise in large quantities in industrial fermenters, a broad range of physical and chemical properties, the possibility of genetic manipulation, and rapid culture development are only a few benefits of using microbial enzymes (Simair *et al.*, 2017; Surovy *et al.*, 2022).

One of the most valuable enzymes used in biotechnology is amylase. Through hydrolysis, it changes starch into glucose. Lipase enzymes are crucial because they hydrolyse acylglycerol into fatty acid and glycerol and influence esterification and transesterification events (Yadav *et al.*, 2022).

These enzymes are known to be produced by a number of microbes, including yeast, bacteria, fungus, and Actinomycetes (Victorino da Silva Amatto *et al.*, 2022; Vijayabaskar *et al.*, 2012). Compared to their plant and animal counterparts, these microbial enzymes are known to be more active and stable (Victorino da Silva Amatto *et al.*, 2022). This encourages the use of microbial enzymes in a variety of industrial applications.

This study, therefore, evaluated the optimization of amylase and lipase produced by *Bacillus cereus* and *B. subtilis* isolated from waste dumpsites.

## .MATERIALS AND METHODS

### Sample collection

Using a soil auger, two- to ten-centimetre-deep soil samples were collected from five designated domestic waste dumpsites in Akure, Ondo State, Nigeria (Table 1).

### Isolation of organisms

The nutrient agar (NA) preparation was done according to standard protocol with modifications (Zhang *et al.*, 2019). The bacterial colony count was conducted using the serial dilution technique. Each soil sample was transferred to a beaker and mixed with 10 cc of distilled water to make a stock solution. Aliquots of each serial fold dilution were poured onto Petri dishes containing nutritional agar using pour plate techniques. Up to $10^{-7}$ of the serial fold dilutions were created. The plates were incubated at 37$^{o}$C for 24 hours and at room temperature for 4 days to produce pure cultures, and colonies were repeatedly sub-cultured.

### Identification of bacterial isolates

Based on their morphological and biochemical traits, such as colour, shape, elevation, consistency, margin, Catalase test, Methyl Red-Voges Proskauer test (MRVP), Sugar fermentation test, Kovacs citrate, indole, and starch hydrolysis, pure cultures of the heterotrophic bacterial isolates were identified (Cao *et al.*, 2022; Rabapane *et al.*, 2022). Molecular identification was carried out according to standard protocol (Tamura *et al.*, 2013).

### Bacterial screening for enzyme production

The bacterial screening was evaluated according to standard protocol with slight modifications. A single colony of bacteria was transferred aseptically from the agar slants into various 100 ml Nutrient broth produced in a 250 ml Erlenmeyer flask. The flasks were incubated in a rotary shaker at 150 rpm for 18 hours at 28$^{o}$C and 28$^{o}$C. The combinations were considered to be seed cultures. Separate basal media (100 ml) containing 0.9 g/L K2HPO4; 0.2 g/L KCl; 0.2 g/L MgSO4.7H2O; 1.0 g/L NH4NO3; 0.002 g/L ZnSO4; 0.002 g/L MnSO4; 0.002 g/L FeSO4.7H2O and 10 g/L dump site sample were created for the isolates (Amin *et al.*, 2022; Rabapane *et al.*, 2022).

The media were autoclaved for 20 minutes at 121$^{o}$C at 15 atm and then allowed to cool. Five millilitres (5 ml) of each seed culture were added to the medium containing the combined sample from all the dumpsites. The culture was incubated at 28$^{o}$C for 36 hours at 150 rpm in a rotary shaker incubator. After the incubation time, 10 ml samples were collected in separate sample vials and centrifuged for 20 minutes at 5,000rpm and 4$^{o}$C. The supernatant obtained after centrifugation was stored at 4$^{o}$C until required, whereas the remaining material was discarded.

### Amylase assay

Precisely, 0.5 mL of the diluted enzyme was added to a tube containing 1.5 mL of potato starch solution at 2% (w/v) and 1 mL of 0.05 M acetate buffer at pH 5.0. The resulting mixture was incubated for 15 minutes at 40°C. Then, 1 ml of the mixture was transferred to a fresh tube containing 1 mL of 3, 5-dinitrosalicylic acid and allowed to stand for 10 minutes in water that had been brought to boiling point. At 540 nm, the colour density was evaluated

**Table 1.** Geographical location (latitude/longitude) of the waste dumpsites in Akure

| Dumpsites | Area/District |
|---|---|
| A | Ago Ireti, Oba Ile (7.26169N/5.22414E) |
| B | Igbatoro road, Imafo (7.21863N/5.21843E) |
| C | Oke Aro road, Eyinke (7.2498N/5.19155E) |
| D | Car street, Eruoba Section C (7.25353N/5.19115E) |
| E | Oba-Adesida, Amudipe elisa (7.25128N/5.20078E) |

spectrophotometrically (Rabapane *et al.*, 2022).

**Lipase assay**

The crude enzyme preparation was used as the culture broth after the separation of cells and particles. The enzyme was stored at 4°C until further use. Lipolytic activity was assayed by the Colorimetric method based on the activity in cleavage of p-nitrophenyl palmitate (p-NPP) at pH 8.0 (Amin *et al.*, 2022).

**Optimization of amylase and lipase Production**

Using the conventional approach, the impact of several parameters on the isolate's ability to produce amylase was standardised with regard to incubation duration, temperature, pH, carbon supply, and nitrogen source and measured at different time intervals and conditions (Amin *et al.*, 2022).

**Effect of incubation period**

The flasks were incubated at 35°C for a variety of times, ranging from 5 hours to 30 hours, following inoculation.

**Effect of temperature**

The effect of temperature on the production of amylase was observed in the nutrient starch broth at varying temperatures (28 °C to 48°C).

**Effect of pH**

The effect of pH was determined by using 0.1M acetate buffer for pH range 3.0-5.0, while 0.1M phosphate buffer was used for pH range 6.0-8.0 to study the effect of pH on both amylase and lipase activities.

## RESULTS AND DISCUSSION

Seven bacterial species including *Bacillus cereus, B. subtilis, Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, Proteus mirabilis, Micrococcus luteus* were identified and tested quantitatively for amylase and lipase enzyme production (Table 2). The present study noticed that two of the isolates namely *B. cereus* strain LA326 and *B. subtilis* strain AU021 were the isolates with the maximum enzyme production and was therefore used for further studies.

As presented in Fig. 2, the effect of the incubation period on the bacterial isolates examined at intervals of six hours revealed *Bacillus cereus* had the highest amylase activity of 76 mmol/min produced at 12 hours of incubation time. Then a steady decline of the amylase activity was observed at 18-, 24-, 30- and 36 hours of incubation time, respectively. This may be a result of the combination of the incubation period's impact on isolate *B. cereus.*

The production of amylase by *Bacillus subtilis* was highest at (68 mmol/min) and 18 hours of incubation time. At incubation periods of 24, 30 and 36 hours, respectively a decrease in amylase activity was observed in *B. subtilis.* At 24 hours of incubation, the amylase activity of both *B. subtilis* and *B. cereus* were observed to be equal at 50 mmol/min enzyme activity as indicated in Fig. 2. This could indicate their similarities in metabolic reactions to environmental factors. Also observed in the study is the effect of the incubation period on lipase activity in *B. subtilis* and *B. cereus.* Both isolates reached their peak lipase activity after 18 hours of incubation (16 mmol/min). At 6 and 12 hours of incuba-

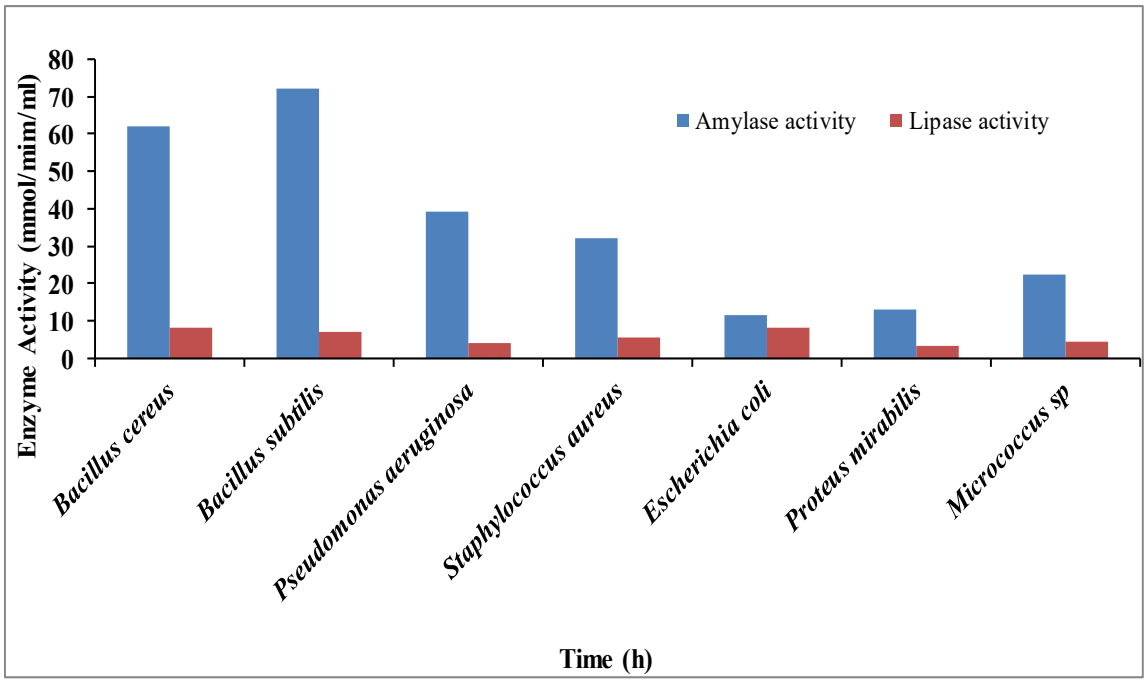


**Fig. 1.** *Quantitative screening of bacterial isolates for enzyme production*

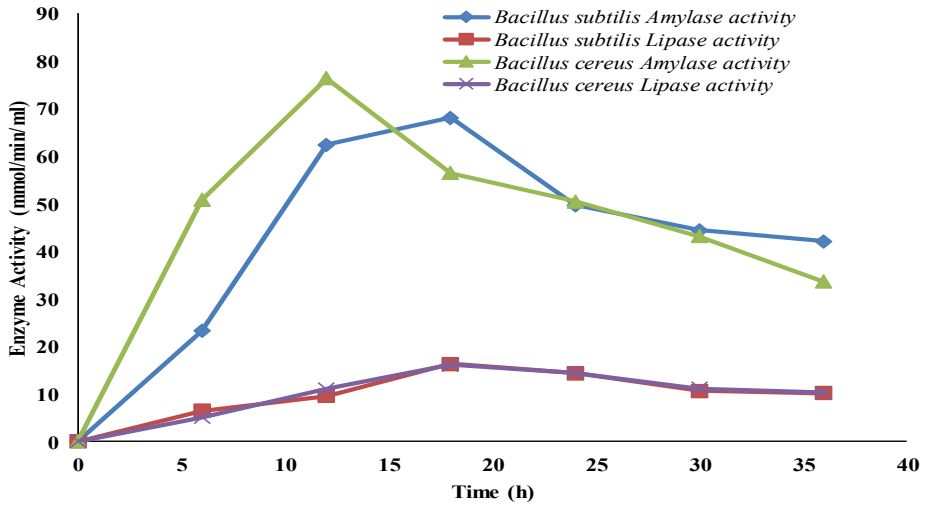


**Fig. 2.** *Effect of incubation period on enzymes production by Bacillus subtilis and Bacillus cereus*

tion time, a steady increase in lipase activity for *B. subtilis* and *B. cereus* was observed.

Yeast extract, ammonium chloride, ammonium nitrate, sodium nitrate, and potassium nitrate were used on *B. cereus* to test the impact of various nitrogen sources on the synthesis of amylase The maximum amylase production was exhibited in yeast extract (52 mmol/min), whereas sodium nitrate had the lowest enzyme production ability (Fig. 3). Also, yeast extract as a nitrogen source produced the highest amylase activity in *B. subtilis*. This could indicate that yeast extract is most suitable as a nitrogen source for optimization of *B. subtilis,* and *B. cereus* isolated from waste dumpsites. In addition, lipase produced by *B. subtilis,* and *B. cereus* was highest when yeast extract was used as a nitrogen source for nutritional optimization.

According to a report, the type of substance and the amount of inducers utilised may have an impact on or deregulate the production of enzymes (Abu Yazid *et al.*, 2017; Bindal *et al.*, 2022). As observed in Fig. 4, nutritional supplements which included glucose, sucrose, maltose, galactose, and rice bran were used as carbon sources on *B. subtilis* and *B. cereus* for lipase and amylase production. The carbon sources used for lipase production by *B. cereus* were observed to be moderately produced while glucose as a carbon source showed the highest lipase activity by *B. subtilis.* Glucose and maltose were observed to be the best carbon source for amylase production in *B. subtilis* and *B. cereus* respectively (Fig. 4).

As presented in Fig. 5, findings in this study showed enzyme activity by *B. subtilis* and *B. cereus were* rec-

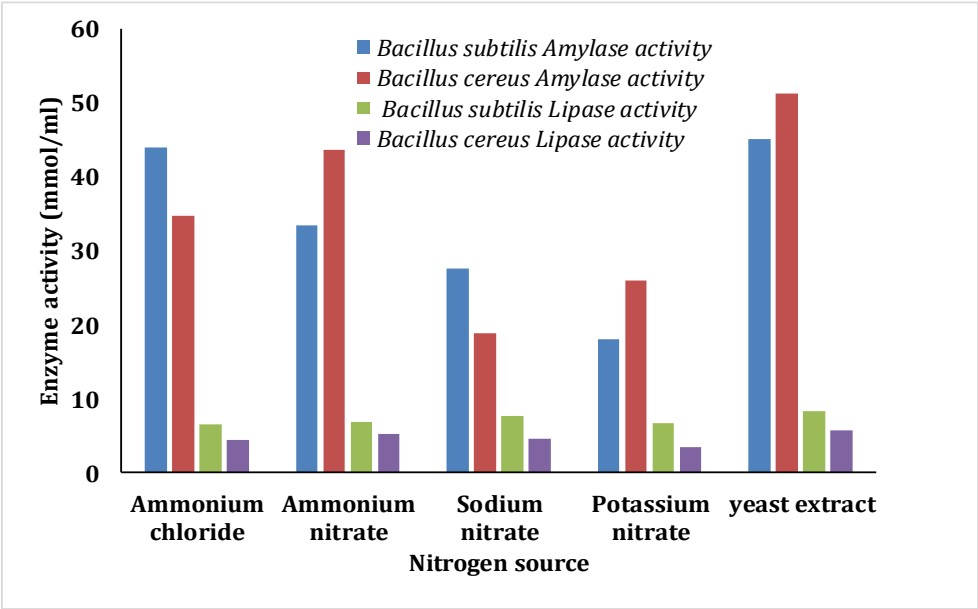


**Fig. 3.** *Effect of nitrogen source on amylase and lipase produced by Bacillus subtilis and Bacillus cereus*

**Table 2.** Morphological and biochemical characteristics of bacterial isolated from dumpsites

| **Characteristics/ Lab Ref.** | **B1** | **B2** | **B3** | **B4** | **B5** | **B6** | **B7** |
|---|---|---|---|---|---|---|---|
| Colour | Creamy | White | Green | Pale yellow | Creamy | Creamy | White |
| Gram's rxn/Shape | +ve Rod | –ve Rod | –ve Rod | +ve Cocci | -ve Rod | –ve Rod | +ve Cocci |
| Catalase | + | + | + | – | + | + | + |
| Motility | + | + | – | – | + | + | – |
| Methyl Red | – | – | – | – | + | + | – |
| Coagulase | – | – | – | + | – | – | – |
| Citrate utilization | + | + | + | + | – | + | – |
| Urea | + | – | + | + | – | + | – |
| Oxidase | – | – | + | – | – | – | + |
| Indole | – | – | – | – | + | – | – |
| Glucose | + | + | – | + | + | + | + |
| Lactose | + | – | – | + | + | – | + |
| Gas | – | – | – | – | + | + | + |
| $H_2S$ | + | + | – | – | – | + | + |
| **Isolate identity** | *Bacillus cereus* | *Bacillus subtilis* | *Pseudomonas aeruginosa* | *Staphylococcus aureus* | *Escherichia coli* | *Proteus mirabilis* | *Micrococcus luteus* |

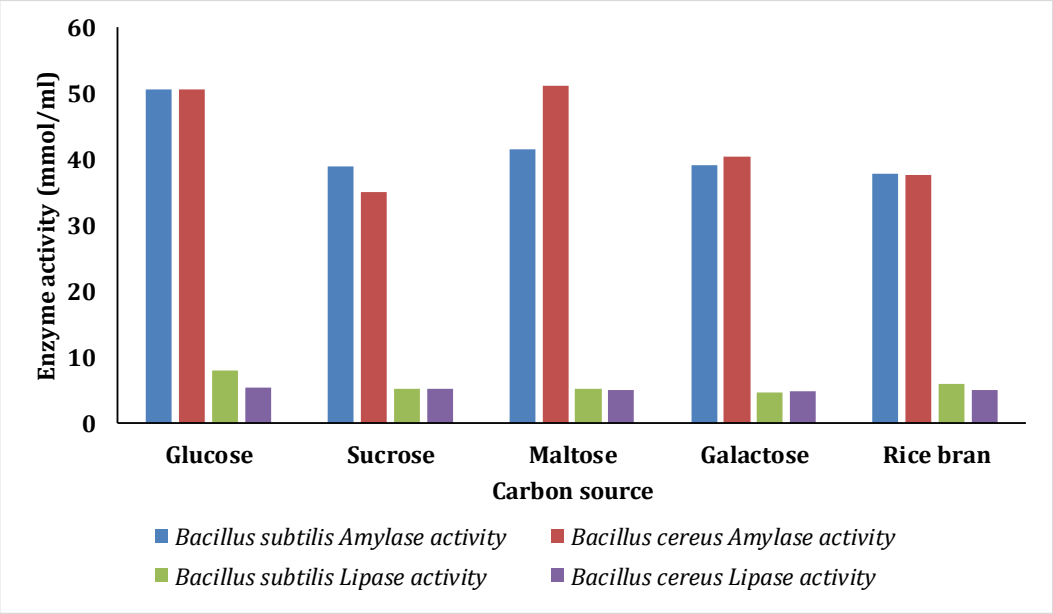


**Fig. 4.** *Effect of carbon source on amylase and lipase produced by Bacillus subtilis and Bacillus cereus*

orded at the optimum temperature of 40°C and 50°C for amylase and lipase respectively. *Bacillus subtilis* was reported as a leading proteolytic bacterium at the optimum temperature of 50°C (Contesini *et al.*, 2018). This could be due to their similarities in physiology and metabolic processes.

Findings in this study revealed, that *B. subtilis* produced the highest amylase at neutral pH of 7 (100% RA). Researchers reported that optimum pH was attained at pH 7 for amylase and lipase enzymes produced by *B. subtilis* and maximum amylase production was achieved at neutral pH (7) (9.00 Units/ml) by *Bacillus* sp. (Nolasco-Soria, 2021). This is in accordance with the findings in this study, as revealed in Fig. 6. The optimum pH for *B. cereus* recorded in this study showed maximum enzyme activity at pH 6 for amylase production and pH 8 for lipase production. Temperature and pH are important factors in enzyme productivity by microorganisms. Amylase is a crucial and essential enzyme produced primarily from microbial sources and used in industrial sectors including Pharmaceutical, Food, Brewing and beverage sectors (Kumar *et al.*, 2014). It is a key player in the field of biotechnology (John, 2017).

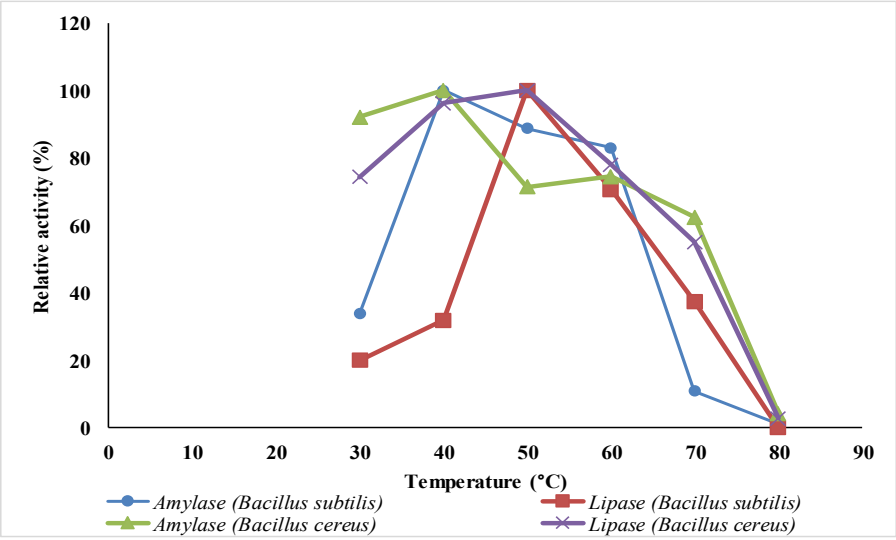


**Fig. 5.** *Effect of temperature on enzymes produced by Bacillus subtilis and Bacillus cereus*

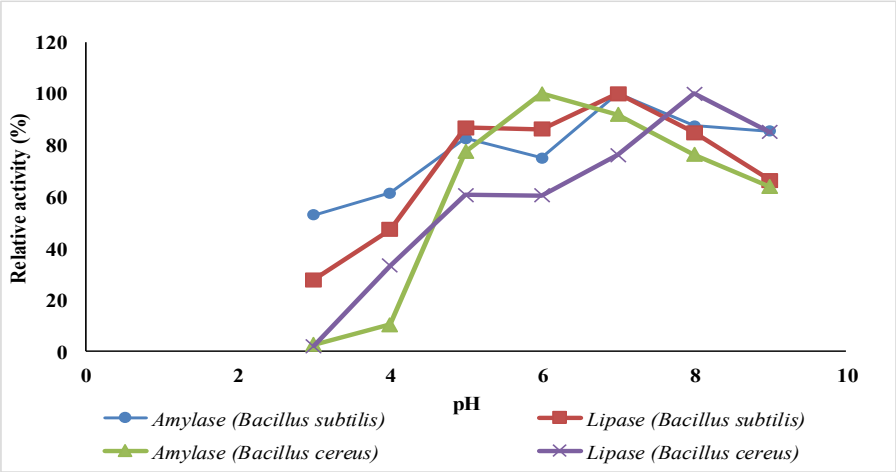


**Fig. 6.** *Effect of pH on enzymes produced by Bacillus subtilis and Bacillus cereus*

## Conclusion

This study revealed that *B. cereus* and *B. subtilis* isolated from waste dumpsites are good producers of amylase and lipase. The enzymes' characteristics depended on the available biomass or substrate concentration acting as a carbon or nitrogen source. Furthermore, these species of bacteria isolates can be harnessed for large-scale production of enzymes and easy degradation of waste material or by using the waste material as a substrate for the growth of microbes.

## Conflict of interest

The authors declare that they have no conflict of interest.

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