ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 6, Issue 2, pp: (89-95), Month: April - June 2018, Available at: www.researchpublish.com

Optimization of Media and Environmental Parameters for Lipase Production by Serratia sp. GMS16

[1]Uduak Camilus Ben, [2]Dominic Reuben Tiku, [3]Godwin Egbe John

[1,2,3]Department of Microbiology, University of Calabar, Calabar.

Abstract: **The study was aimed at optimizing the production of lipase using media and environmental parameters. Serratia GMS16 was isolated from a domestic waste dump soil sample, and evaluated for lipase producing capability. Media (carbon, nitrogen) and environmental (pH, temperature, and fermentation time) parameters were investigated for maximum lipase production. Results obtained revealed that olive oil was the best carbon source for the elaboration of lipase by the selected bacterium, however spent frying oil was adopted for production from the viewpoint of production economics and raw material availability. The inclusion of proteose peptone in the medium resulted in high lipase production. Enzyme activity was observed to increase with increasing fermentation time, at least within the ambience of the total fermentation time of 72h and at a pH of 7.0. Analysis of variance (ANOVA) of data and regression analysis showed a significant (P = 0.000; R2 = 0.958) 95.8% of final lipase concentration of 90.74 µg/mL was obtained from the interactive effects of the input variables used in the experiment.**

1. INTRODUCTION

The advent of enzymology represents an important breakthrough in the biotechnology industry, with the worldwide usage of enzymes being nearly U. S. $1.5 billion in 2000 (Kirk et al., 2002). The major share of the industrial enzyme market is occupied by hydrolytic enzymes, such as proteases, amylases and lipases. In recent times, lipases have been of great importance within biotechnological perspectives (Ire et al 2015). Many attempts have been made to isolate lipase-producing microorganisms since this enzyme is used in numerous biotechnological processes including, food, leather, cosmetics, detergents, pharmaceuticals and industrial waste management (Freire et al 1997). Lipases are acidic glycoproteins with the biological function of catalyzing the hydrolysis of triacylglycerol to diacylglyceride, monoacylglyceride, glycerol and long-chain fatty acids. Bacterial lipases are mostly extracellular and their activities are greatly influenced by nutritional and physicochemical factors such as temperature, pH, nitrogen and carbon sources, inorganic salts, agitation and incubation time. Additionally, lipases can carry out reactions under mild conditions of pH and temperature and this reduces energy needs to direct reactions at unusual temperatures and pressures (Jigita et al., 2011). Due to the specificity of lipases, the production of unwanted products in the waste stream are decreased or eliminated. Thus, it can be said that lipases are environmental friendly. Lipases have been used therapeutically and industrially for a long time. In developing countries such as Nigeria, lipases are used in food, detergent, cosmetic, pulp and paper, leather and pharmaceutical industries are imported from other countries. These imported lipases are expensive and difficult to access, thus, leading to a decrease in the production of these products. In view of this, there is a great urge to locally bio-prospect for lipase-producing bacteria for industrial use (Sirisha et al., 2010).

2. MATERIALS AND METHODS

Microorganism

Serratia sp. GMS16 was used for this investigation. It was isolated from soil samples collected from Goldie market in Calabar South Local Government Area, Cross River state, Nigeria.

Lipase assay in solid and submerged media

The liquid culture medium containing (g/L) K2HPO4 1.0, KH2PO4 0.5, MgSO4.7H2O 0.2, FeSO4.7H2O 0.01, NaCl 2.0, NH4Cl 1.0, 1% tween 80 (Ekpenyong et al., 2017) was used for lipase production. For the production of lipase, 250 mL Erlenmeyer flasks containing 50 mL of sterile medium was inoculated with a 1 mL aliquot of an 18-h old Luria broth culture of Serratia sp. GMS16. The inoculated flasks were then incubated for a period of 72 h at room temperature on a rotary shaker at 150 rpm. After 72 h of incubation, the culture was centrifuged twice at 4,000 rpm for 20 min and the cell-free culture supernatants were assayed for the presence of lipase by the Bradford method (Bradford, 1976). For the solid medium, the pure culture was inoculated in triplicates by means of line streak on the same medium as described above but solidified with 1.5% agar-agar. Inoculated plates were incubated for 72h at room temperature. After incubation, diameters of precipitation zones and bacterial growth were measured by means of meter rule and then used to calculate the productivity using the formula P=TPZ - CD/CD X 100. Where; P= productivity (%), TPZ = Total precipitation zone (cm) and CD = colonial diameter (cm).

Protein Detection and Quantification by the Bradford Method

This followed the description by Bradford (1976) using bovine serum albumin (BSA) as standard. Various concentrations (5 to100 µg) of the standard protein, bovine serum albumin, obtained from Nutritional Biochemicals Coorporation were prepared in 0.15 M NaCl.

The protein reagent, Coomassie Brilliant Blue G-250 was prepared by dissolving 100 mg of it in 50 mL 95% ethanol. To this solution 100 mL 85% (w/v) phosphoric acid was added. The resulting solution was diluted to a final volume of 1 L.

Protein solution containing 5 to100 µg proteins in a volume up to 0.1 mL was transferred into 13 x 125 mm test tubes. The volume in each test tube was adjusted to 0.1 mL with phosphate buffer (0.05 M; pH 7.0). Five milliliters (5 mL) of protein reagent was added to each test tube and the contents mixed by inversion. Development of blue color was indicative of protein presence. The absorbance at 595 nm was measured after 2 min in 3 mL cuvettes against a reagent blank prepared from 0.1 mL of phosphate buffer (pH 7) and 5 mL of protein reagent for intensity quantification. The weight of protein was recorded and later used to plot against the corresponding absorbance resulting in a standard curve used to determine the concentration of protein in the unknown samples. Thereafter, 0.1 mL of sterile enzyme solution was transferred into 13 x 125 mm test tubes and the volume adjusted to 0.1 mL with the phosphate buffer (pH 7). One milliliter of protein reagent was added to the test tubes and the contents mixed as in the standard method. Development of blue color as in the standard indicated protein presence in the crude enzyme broth. Absorbance at 595 nm was measured as in the standard method against a reagent blank prepared from 0.1 mL of the appropriate buffer. Amount of protein in crude enzyme solution was read off and the standard curve prepared as described earlier.

3. OPTIMIZATION OF MEDIA PARAMETERS FOR PROFOUND ENZYME ACTIVITY

Effect of carbon source on lipase production

The selected bacterium was cultured in media containing (g/L) K2HPO4 1.0, KH2PO4 0.5, MgSO4.7H2O 0.2, FeSO4.7H2O 0.01, NaCl 2.0, NH4Cl 1.0 77with pH adjusted to 7.0 with 0.5 M NaOH and 0.5 M HCl. One per cent (1 % v/v) of each of Tween 80, olive oil and spent frying oil was added into culture media in test flasks as carbon source. Minimal medium in control flask was supplemented with glucose (1 % w/v) as carbon source. All flasks were prepared in duplicates and incubated at room temperature. The culture broth was harvested at 48 h by centrifugation at 4,000 rpm for 20 min and supernatant used as crude enzyme solution. Enzyme detection was carried out using the Bradford method and amounts obtained by comparison with the standard curve.

Effect of nitrogen source on lipase production

To evaluate the effect of various nitrogen sources on lipase production by the choice bacterium, different nitrogen sources including urea, proteose peptone, asparagine, beef extract and yeast extract were added at 1 % concentration to the minimal medium used in the previous section with spent frying oil as carbon source. One per cent (1 % w/v) NH4Cl was used in the control flask. All preparations were made in duplicates and flasks incubated at room temperature with agitation at 150 rpm for 48 h. The culture broth was harvested by centrifugation at 4,000 rpm for 20 min. The supernatant was used as crude enzyme solution to assay the amount of enzyme produced by comparison with the standard curve prepared.

Optimization of Environmental Parameters on Lipase Production

To evaluate the effect of the different environmental parameters (pH, temperature and fermentation time) on lipase production by the choice bacterium, the different parameters were maintained at a constant except the one being studied.

The effect of pH on lipase production was studied by cultivating the isolate in different pH values (pH 5-9). 0.1ml phosphate buffer was used.

The effect of temperature was studied by incubating the culture at various temperatures ranging from 25-45℃ on a rotary shaker at 150rpm while keeping the other parameters constant.

To evaluate the effect of fermentation time on lipase production, the organism was cultured in a minimal medium used in the previous section. The culture broth was harvested at 12h interval for 96h.

The culture broths were harvested by centrifugation at 4,000 rpm for 20 min. the supernatants were then used as the enzyme solution to assay the amount of enzyme produced.

4. RESULT AND DISCUSSION

Enzyme assay of Serratia sp. GMS16 in solid and submerged media

The result of productivity potential of the isolate on solid medium revealed a production of 16.67% of lipase. To confirm the bacterial lipase productivity tests on solid media, the experiments were repeated in liquid media and the result revealed that the bacterial isolate scored a visual turbidity of '+++' and produced an absorbance of 0.896 when intensity of blue colour was assessed spectrophotometrically.

Effect of carbon source on lipase production by Serratia sp. GMS16

Carbon sources provide the skeleton of metabolites and bacterial cells, this makes it a key nutritional factor that affects lipase expression. Five different substrates were tested to study their effect on the lipase production. All the substrates enhanced lipase production with particularly high values obtained with olive oil, followed by spent frying oil, tween-80 and sun-flower oil as presented in Figure 2.

Figure 2 reveals that olive oil proved to be the best substrate with enzyme activity of 48.19 µg/mL. This corresponded to an optical density (OD595) value of 1.286 when calculated from the regression equation obtained from the standard curve of Figure 1. This result is in agreement with the previous study conducted by Nwachukwu et al., (2017), where olive oil was preferred by Serratia marcescens for lipase production. Freire et al. (1997) also reported maximum lipase activity from penicillium species when olive oil was used as the carbon source. Similar lipase activity was reported by Serratia rubidaea and staphylococcus epidermidis, which was induced by gingelly oil (Immanuel et al, 2008) and cod liver oil (Esakkiraj et al, 2010), respectively. Bora and Kalita (2002) also observed that lipase productivity by Bacillus sp. DH 4 was higher when cultured in medium supplied with vegetable oils. Spent frying oil was adopted for the production over olive oil based on the viewpoints of production economics and raw material availability.

Effect of nitrogen source on lipase production by Serratia sp. GMS16

Nitrogen supplement is a significant parameter for the growth of microorganisms as well as high lipase production by microorganisms. A range of inorganic and organic nitrogen sources were tested to evaluate their capacity to support lipase production when grown on minimal medium supplemented with spent frying oil as carbon source is presented in Figure 3. Among the tested nitrogen sources, lipase production was the highest in medium supplied with peptone, with beef extract, ammonium chloride and yeast extract yielding the next highest production levels. This revealed that lipase production was higher on organic nitrogen than inorganic nitrogen. Ruche et al, (2008) reported that lipase production by Pseudomonas aeruginosa was specifically higher on organic nitrogen sources than on inorganic sources, which is in agreement with the present study. This is in contrary with the previous study conducted by Ire et al. (2015), where ammonium chloride was reported to be the best source of nitrogen when supplemented with sucrose as the carbon source. In another study, peptone was the preferred nitrogen source for C. albicans (21.10u/ml) and A. flavus (22.4U/ml).

Effect of pH

The initial pH of the growth medium is one of the important environmental parameters that affects lipase production, reason being that enzymes of microbial origin remain metabolically actuve at a favourable pH range and any drastic

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change of the media pH may result in the disruption of microbial membrane transport mechanism leading to loss in enzyme activity. (Jigita et al., 2011).

The result of the effect of pH on lipase production by Serratia sp. GMS16 when grown on the minimal medium supplemented with spent frying oil as carbon and peptone as nitrogen source is presented in Figure 4. The figure shows that the best pH for lipase production is 6-8, where the organism released maximum lipase of 49.62 µg/mL at pH 7.0. this is in correlation with Sujatha et al., 2013 who reported maximum lipase activity of 19.32 U/ml by Pseudomonas aeruginosa KDP. the result is in contrast with the previous study conducted by Jigita et al., (2011), Pseudomonas aeruginosa and A. Flavus produced a maximum amount of enzyme at pH 6.2 and C. albicans at pH 5.2.

Effect of Temperature

The investigation of optimum temperature for lipase production is shown in Figure 5. The figure shows that peak enzyme activity was obtained at 35℃ with a protein concentration of 51.78µg/mL. This result is similar to that of Siriba et al. (2010), where maximum activity was observed at 36℃. In variance with our study, Sujatha and Dhandayuthapani (2013) reported that the maximum lipase activity of 21.45U/ml was obtained at 45℃

Effect of fermentation time

The lipase production by Serratia sp. GMS16 was optimized and assayed as shown in figure 6. The optimum lipase activity 57.18µg/mL was detected at 72h fermentation time. This suggests that the level of enzyme activity increases with increasing fermentation time up-to 72h, thereafter, there was a decrease in the lipase yield after a prolonged incubation time. Jigita et al., (2011) obtained a maximum lipase activity of 19.40U/ml from Aspergillus flavus when incubated for 5days.

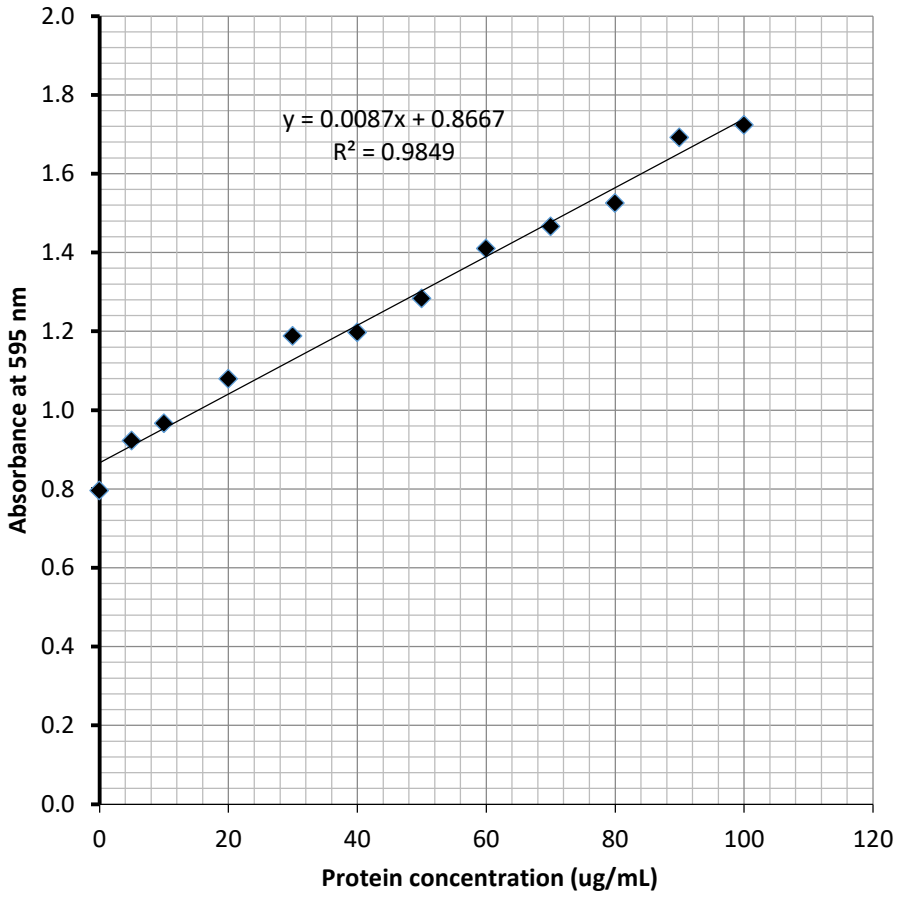

FIGURE 1: Calibration curve for enzyme quantification in crude lipase solution using bovine serum albumin as standard

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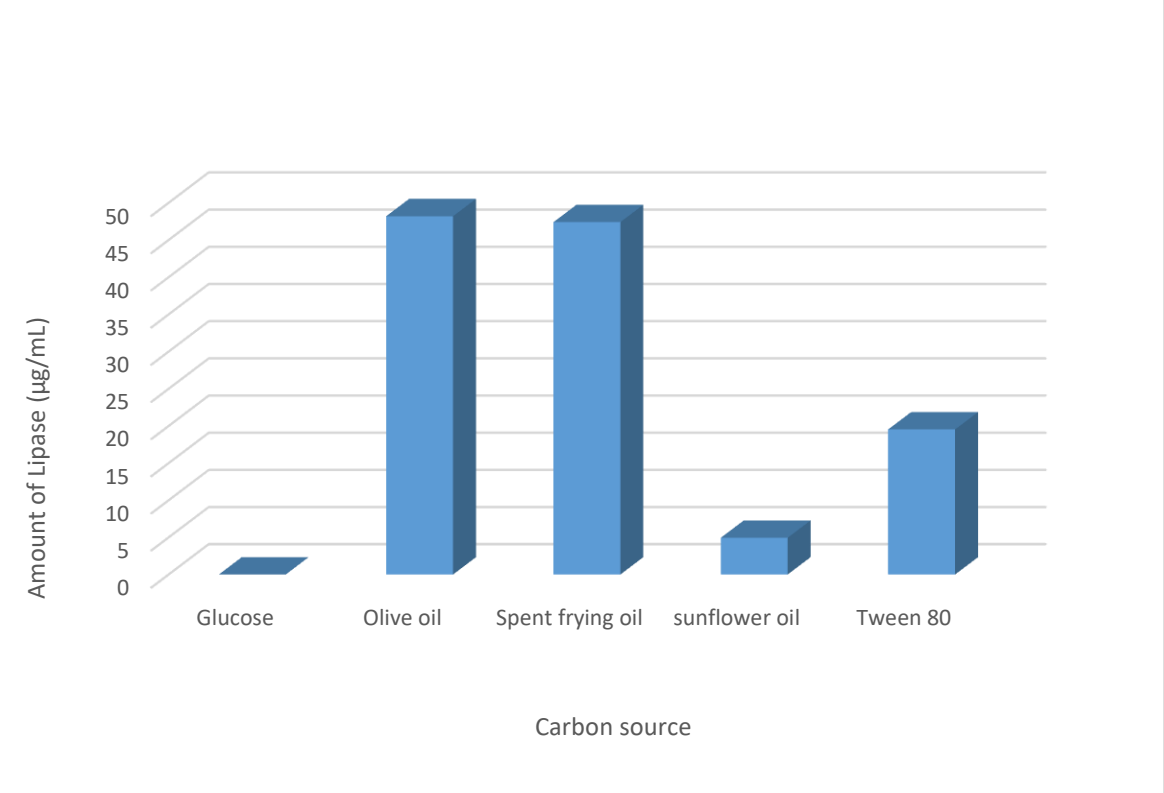

FIGURE 2: Effect of carbon source on lipase production by Serratia sp. GMS16

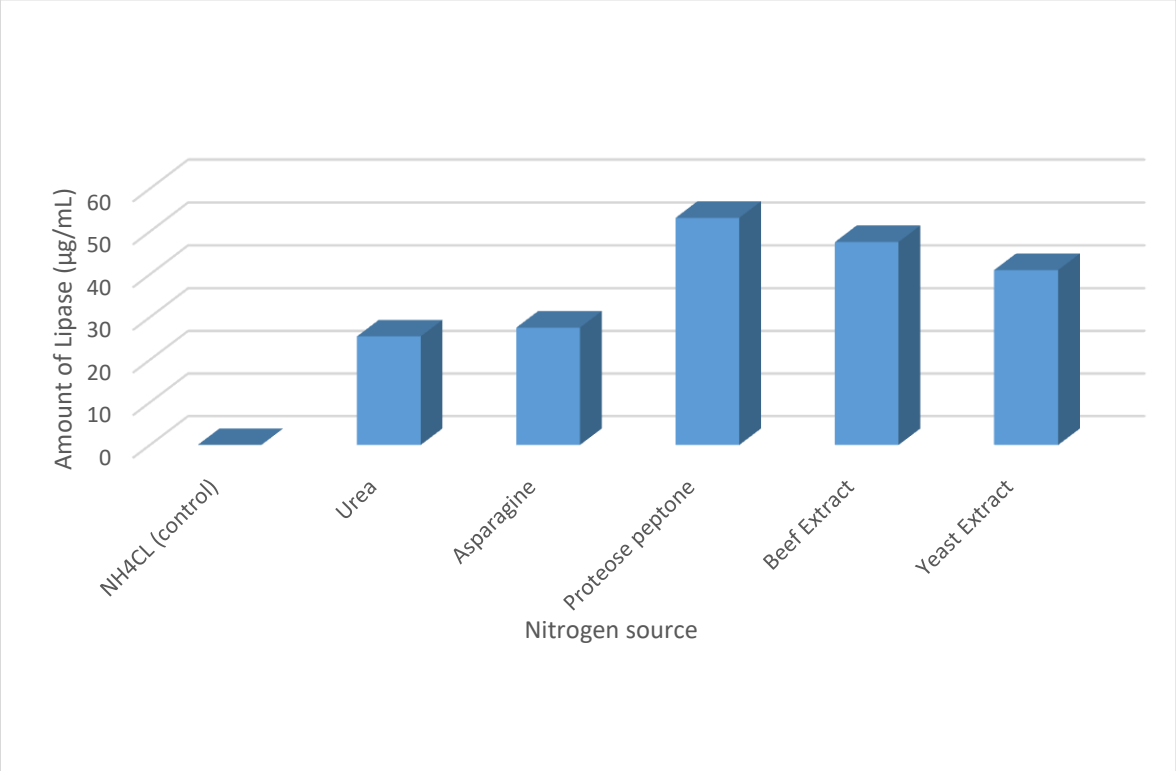

FIGURE 3: Effect of nitrogen source on lipase production by Serratia sp. GMS16

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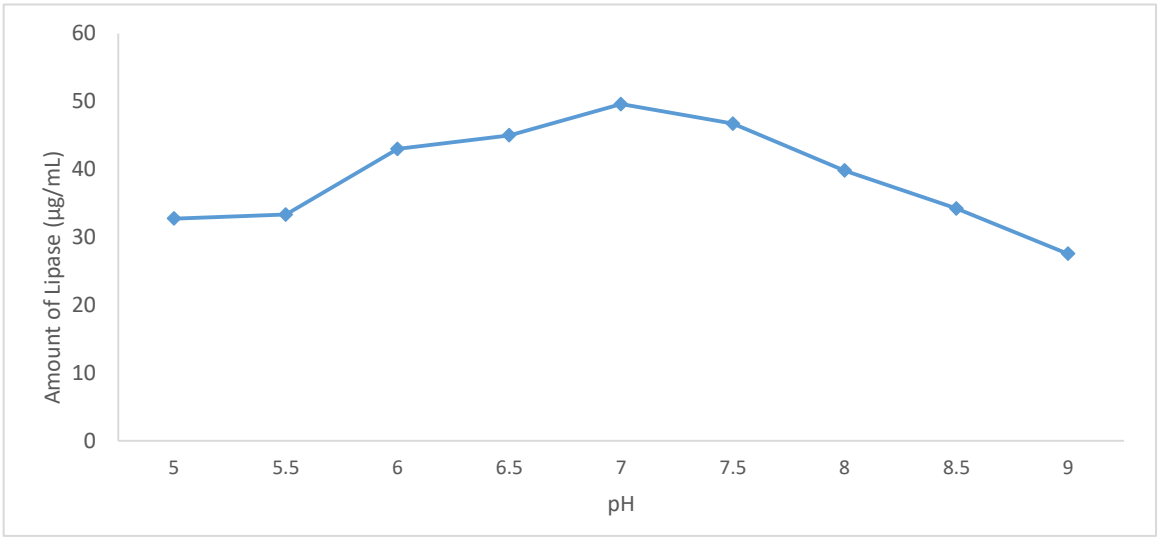

FIGURE 4: Effect of pH on Lipase Production by Serratia sp. GMS16

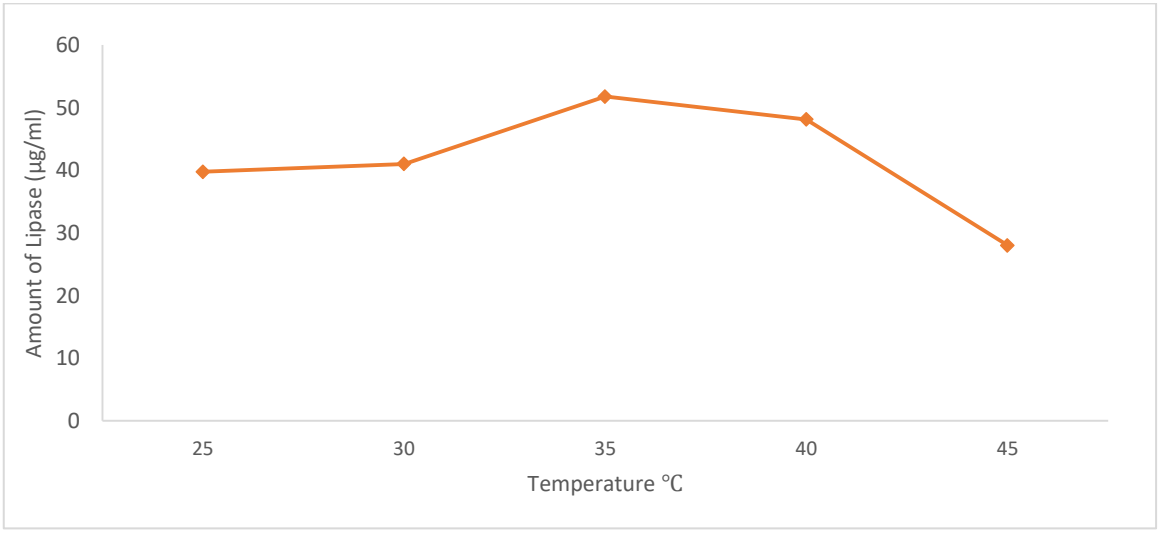

FIGURE 5: Effect of Temperature on lipase Production by Serratia sp. GMS16

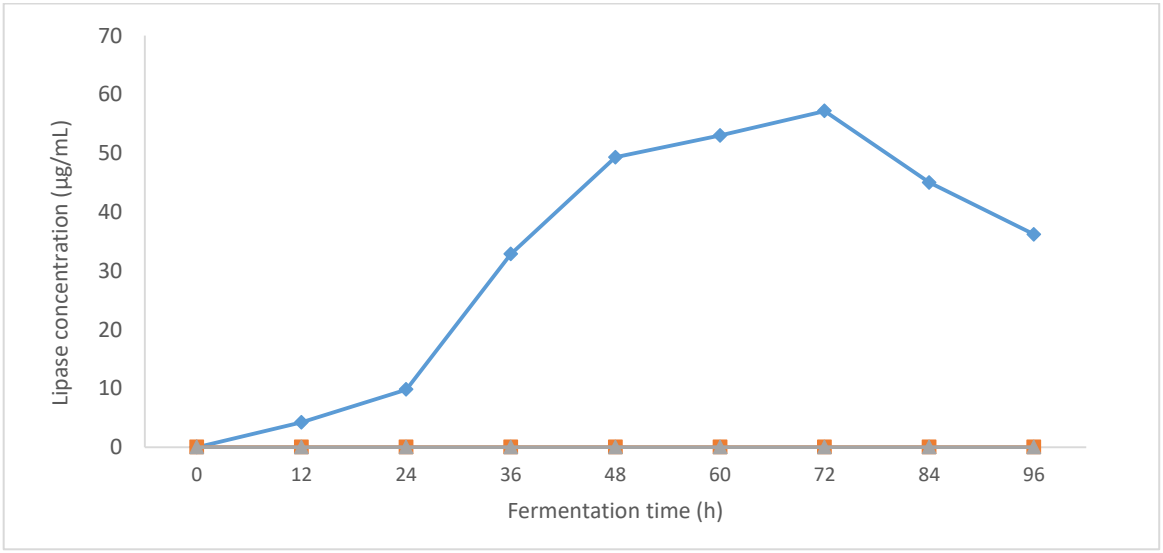

FIGURE 6: Effect of fermentation time on lipase activity by Serratia sp. GMS16

5. CONCLUSION

Serratia sp. GMS16 isolated from Nigerian soil produced commendable amounts of lipase in the presence of spent frying oil and proteose peptone as carbon and nitrogen sources respectively. Interestingly, optimum lipase activity of 90.74µg/mL was attained after 4 days at a temperature of 35℃ and pH 7.0. The adoption of spent frying oil for large scale production makes the process economically feasible.

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