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Optimization of fermentation conditions for the production of α-amylase by *Penicillium* sp. isolated from forest soil

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Abstract

Starch degrading amylolytic enzymes are most important in the field of industrial microbiology and biotechnology with a huge application in food, fermentation, textile and paper industry. A study was conducted to exploit *Penicillium* sp. for α-amylase production. In the study *Penicillium* sp. was found to be a promising producer of α-amylase. Different fermentation conditions for the production of α-amylase from *Penicillium* sp. were optimized. The optimum production was found at initial pH- 7.0, incubation temperature- 30°C, carbon source- Starch (1.5%), organic nitrogen source- Peptone (0.6%), inorganic nitrogen source- $(NH_4)_2SO_4$ (0.02%) and inorganic salt - KH_2PO_4 (0.3%) respectively. The study suggests that the mold *Penicillium* sp. showing 55 hours of optimum incubation time has the potentiality to be an economically viable organism for commercial production of α-amylases.

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Introduction

α-amylases are one of the industrially important enzymes that hydrolyse 1-4 linkages of starch. Sugar, textile, alcohol, detergent, paper and food processing industries such as baking, brewing, production of cakes, fruit juices, starch syrups, preparation of digestive aids, etc. are the major industries where extensive use of α-amylases have been reported (Gupta *et al.*, 2003; Sivaramakrishnan *et al.*, 2006; de Souza and Magalhaes, 2010; Singh *et al.*, 2022). However, the rapid progress in the field of microbial biotechnology, the applications of α-amylases have also been extended in many new areas such as clinical, medical and analytical chemistry (Das *et al.*, 2011; De Souza and Magalhaes, 2010; Far *et al.*, 2020). It was estimated that about 25-30% of the world's enzyme market was occupied by amylases in the beginning of 21st century (van der Maarel *et al.*, 2002; Mostafa *et al.*, 2023). Microbial enzymes have multitude of applications in diverse industries such as textiles, leather, paper and pulp, research and development, pharmaceutical, agriculture, detergent, waste, biorefineries, food and feed industries making them very crucial in industrial production processes (Binod *et al.*, 2013; Okpara, 2022).

α-amylase is found universally throughout the plant, animal and microbial kingdoms. However, the microbial sources, particularly thermophilic bacteria and mesophilic molds provide the industrial demand of α-amylases (de Souza and Magalhaes, 2010; Sivaramakrishnan *et al.*, 2006; Mostafa *et al.*, 2023). The advantages of using microorganisms for the production of α-amylases are the bulk production capacity at industrial scale and the microbes are easy to manipulate to get enzyme of desired characteristics (de Souza and Magalhaes, 2010; Gupta *et al.*, 2003; Singh *et al.*, 2022). Although many microbial species are reported to produce α-amylase, only a few species of *Bacillus* and *Aspergillus* along with their improved strains have been used for the commercial production of α-amylases. Bacteria-sourced alpha-amylase baking enzymes dominated the market and accounted for a share of 68.27% in 2023. Bacterial α-amylase accounted for 83.2% of the global volume in 2015 and the trend is expected to continue over the projected period owing to its high thermal stability as compared to other sources (Grandview research, 2024). The market size of Alpha-Amylase was estimated as USD 10.53 Billion in 2023 and is expected to grow at USD 17.54 Billion by the end of 2030 with a compound annual growth rate (CAGR) of 7.57% for the period 2024-2030 (Verified Market Reports, 2024). Moreover, several *Penicillium* species are also studied by various workers for amylase producing ability and it has been found that species like *P. chrysogenum, P. expansum, P. janthinellum* etc. have great potentiality as commercial producer of amylases (Erdal and Taskin, 2010; Sindhu *et al.*, 2011; Vidya *et al.*, 2012). Due to the widely acknowledged GRAS (generally recognized as safe) status, amylases from fungi are more preferable (Gupta *et al.*, 2003; Ayansina *et al.*, 2017).

Due to increasing need for these enzymes in various industries, the importance of production of enzymes with better properties such as raw starch degrading enzymes suitable for industrial applications and their cost effective production techniques is also expanding (Sivaramakrishnan *et al.*, 2006; Ayansina *et al.*, 2017). In 2023, the dominated application of amylases was observed in bread and bakery industry. In terms of region, the α-amylase market was dominated by North America with 37% share in 2023. Asia Pacific is anticipated to witness significant growth in the global market since the region has a large and growing population which will contributing to increased consumption of baked goods. The demand for alpha-amylase enzymes rises correspondingly to meet the needs of the expanding market (Grandview research, 2024). The enzymes market size in India was valued at USD 440.5 million in 2023 which is expected to rise at a CAGR of 7.8% during 2024 to 2030 (Grandview research, 2024). Therefore, there is always a search for new and better source of microorganism for amylase production. Since, the North-East India including Assam harbours a high biodiversity, it is necessary to bio-prospect the microbial resources for various industrial applications. Moreover, no such extensive investigations have been reported from this region.

Thus, the study aimed to exploit microorganism particularly *Penicillium,* with better amylase production competence.

Materials and methods

Microorganism

The experiment was done by using *Penicillium* sp. as inoculum which was isolated from soil samples collected from Chakrashila Wildlife Sanctuary, Kokrajhar, Assam, India. The strain was maintained on potato dextrose agar slants at 4°C and regularly sub-cultured at an interval of 30 days.

Amylase production under submerged fermentation

Spores of *Penicillium* sp. were harvested from seven day old slant culture as suspension in sterile distilled water containing 0.01% Tween-80. The spores were dislodged using a sterile inoculation needle under aseptic conditions. The spore suspension was diluted to desired count (5×10^7 spores/ml) that served as inoculums. Submerged fermentation was carried out in 250 ml Erlenmeyer flask by using a culture medium containing (g%) KH_2PO_4 1.5, $MgSO_4.7H_2O$ 0.5, $FeSO_4$ 0.01, Yeast extract 3, Peptone 5, Starch 20. For fermentation 50 ml of sterile culture medium was taken in flask. After inoculation, the flasks were incubated at 30°C for 70 hours under stationary conditions in a BOD incubator. In the end of the fermentation, the flasks were shaked for 15 min and then filtered through whatmann No. 1 filter paper. The filtrate was used as crude enzyme extract (source of amylase).

Optimization of process parameters for α-amylase production

The objective of the study was to examine the optimum conditions for the production of extracellular amylase from *Penicillium* sp. The effect of different pH, incubation temperature, different starch concentrations, additional carbon sources, organic nitrogen sources, inorganic nitrogen sources and different inorganic salts are studied using a medium containing (g%) KH_2PO_4 1.5, $MgSO_4.7H_2O$ 0.5, $FeSO_4$ 0.01, Yeast extract 3, Peptone 5, Starch 20. All the parameters were examined separately. For each studied parameter 3 sets of experiment were performed.

Effect of incubation time: The effect of incubation time on α-amylase secretion was studied by incubating the flasks at for 40 to 70 hours.

Effect of initial pH on α-amylase production: The effect of initial pH on α-amylase yield by *Penicillium* sp. was studied by adjusting the pH of the medium to various pH levels (pH: 4.0 to 8.0).

Effect of temperature: The effect of temperature on enzyme production was determined by incubating the flasks at different temperatures (25 to 40°C).

Effect of starch concentration: The effect of different concentrations of starch (0.5 to 2g/l) in the production medium was studied for *Penicillium* sp.

Effect of different additional carbon sources: The effect of additional carbon sources on amylase production was studied by adding 1% level in the medium.

Effect of different organic nitrogen sources: The effect of different concentrations of different organic nitrogen sources in the medium was evaluated. The different concentrations were 0.2 to 0.7 g/l (w/v).

Effect of different inorganic nitrogen sources: Different inorganic nitrogen sources (0.02 to 0.1%; w/v) such as ammonium chloride, ammonium sulfate, ammonium nitrate, sodium nitrate and urea were evaluated for the enzyme production.

Effect of different inorganic salts: The effect of different inorganic salts such as KCl, NaCl, $FeSO_4$, $MgSO_4$, KH_2PO_4 and K_2HPO_4 were evaluated for α-amylase production.

Measurement of amylase activity

The culture was filtered through Whatmann No. 1 filter paper and the filtrate was used to measure the amylase activity. The procedure was as follows-

A reaction mixture comprising 1 ml of enzyme extract and 1 ml of substrate (i.e. 1% soluble starch solution) was added in test tube and incubated at 50°C for 30 min. Then the reaction was stopped by the addition of 3ml of DNS reagent and boiled for 10 minutes. It was allowed to cool at room temperature and the absorbance was measured using spectrophotometer at 540nm (Miller, 1959). A standard graph was prepared with 0-100 µg maltose. The amylase activity of the test samples were measured from the graph. One unit (IU) of α-amylase activity is defined as the amount of enzyme which releases 1 µg of reducing sugar per minute under the standard operational conditions.

Statistics

All the experiments were done as triplicate and results show the mean values (with standard deviation).

Results and discussion

Effect of incubation time

The results shown in Fig. 1 depict that the highest production of α- amylase i.e., 60.12 units/ml was recorded at 55 hours of incubation. It is unlike the results of other researches such as Tiwari *et al.* (2007), Premalatha *et al.* (2022), etc. where optimum incubation time was 72 to 96 hours. This may be a very important criterion for producing amylase from fungal sources which will reduce the time of incubation to get highest production.

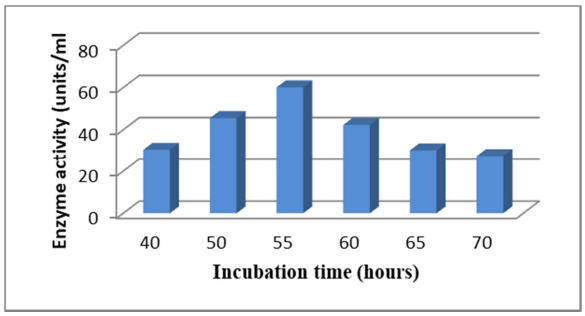

Fig. 1. Effect of incubation time on amylase production

Effect of initial pH

Initial pH of the medium plays an important role in growth and product formation. The results presented in Fig. 2 show that the maximum amylase was produced at pH 7.5 (65.81 units/ml). However, Tiwari *et al.* (2007) reported optimum initial pH of 7.0 and Vidya *et al.* (2012) reported optimum initial pH for amylase production was 6.0. The pH optima differ for different organisms. Below or above the optimal level, drop in the production of amylase was evidenced. The reason is that enzymes act more efficiently within a narrow range of pH and is very sensitive to a little change in pH (Abdullah *et al.*, 2017).

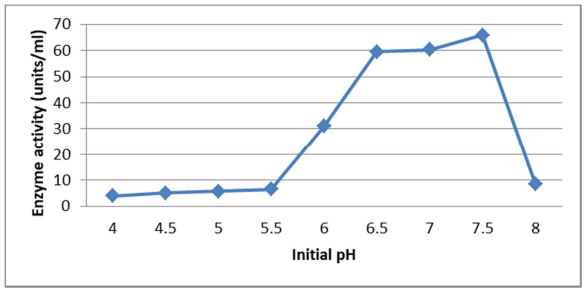

Fig. 2. Effect of initial pH on amylase production

Effect of temperature

Submerged fermentation is generally carried out in the temperature range of 25-35°C and the optimum temperature for amylase production is different for different species (Sivaramakrishnan *et al.*, 2006; Abdullah *et al.*, 2017; Balakrishnan *et al.*, 2021). It was observed that the optimum temperature in the experiment was 30°C (Fig. 3). It was noted from the observations that at higher temperatures than the optimum i.e., 30°C, the production of amylase decreases and at 40°C the amylase production is the least (5.1 units/ml). Moreover, the mycelia growth was also minimum at 40°C.

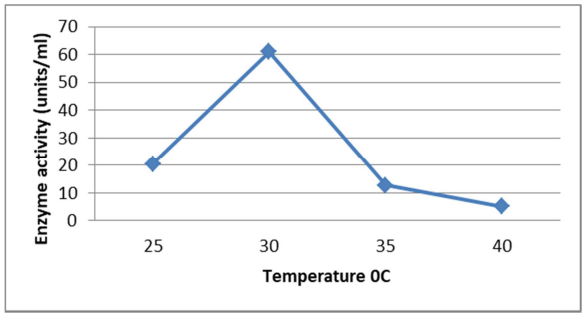

Fig. 3. Effect of incubation temperature on amylase production

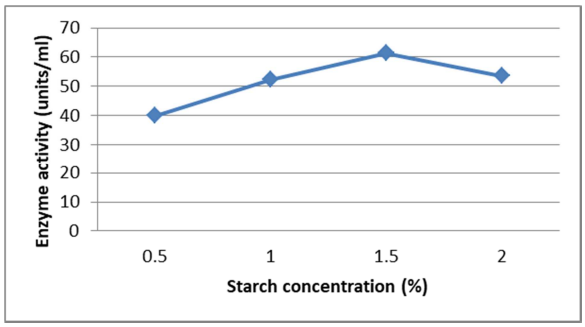

Fig. 4. Effect of different concentrations of starch on amylase production

Effect of starch concentration

The effect of different concentrations of starch (5 to 20g/l) in the production medium was studied for *Penicillium* sp. It was found that 1.5% starch concentration showed highest production of α-amylase (61.42 units/ml) during the study (Fig. 4). Starch concentration also determines the amount of α-amylase produced by microorganism. α-amylase production is induced by starch, however, only a certain concentration gives highest production.

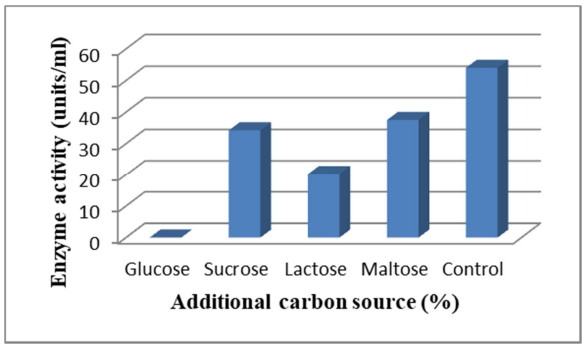

Fig. 5. Effect of different carbon sources on amylase production

Effect of different additional carbon sources

The effect of different carbon sources was studied on the production of amylase by *Penicillium* sp. during the investigation. Glucose, sucrose, lactose and maltose were used as additional carbon sources in the medium besides starch. It was found that presence of additional carbon in the medium inhibited α-amylase production (Fig. 5). Glucose in the culture medium showed complete inhibition of α-amylase production since no amylase was detected. Probably additional carbon sources in presence of starch acted as catabolic repressor and resulted in reduction in enzyme production. As amylase is an inducible enzyme, starch stimulates the production of amylase and glucose represses the production (Gupta *et al.*, 2003; Sivaramakrishnan *et al.*, 2006; Abdullah *et al.*, 2017).

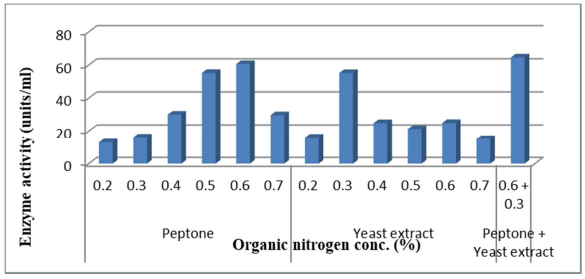

Fig. 6. Effect of organic nitrogen sources on amylase production

Effect of different organic nitrogen sources

The amylase synthesis in presence of complex organic nitrogen sources by *Penicillium* sp. was studied. Peptone, yeast extract and combination of peptone and yeast extract in various concentrations were examined for amylase synthesis (Fig. 6). It was observed that both peptone and yeast extract alone in the medium can yield considerable amount of amylase i.e., 60.63 units/ml and 55.21units/ml respectively. Peptone is considered as ideal source for amylase synthesis and favours the growth and product formation (Tiwari *et al.*, 2007; Premalatha *et al.*, 2022). However, when peptone and yeast extract were applied together in the medium, α- amylase production increased to 64.63 units/ml. It is higher than when each was applied separately.

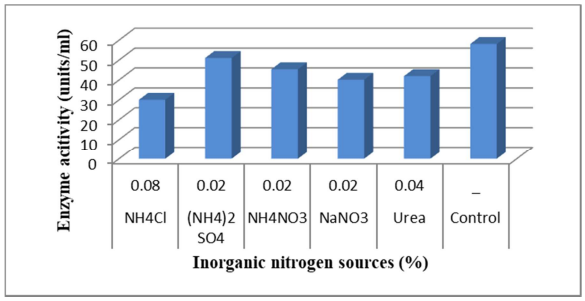

Fig. 7. Effect of inorganic nitrogen sources on amylase production

Effect of different inorganic nitrogen sources

The effect of inorganic nitrogen sources for amylase production by *Penicillium* sp. was also studied

separately from the organic nitrogen sources. NH_4Cl, NH_4SO_4, NH_4NO_3, $NaNO_3$ and Urea were added in the medium at different concentrations (Fig. 7). The observations showed that all inorganic sources were though able to induce the production of amylase but could not substitute organic sources fully in a medium for amylase synthesis. This observation is supported by the findings of other workers like Sindhu *et al.*, 2009; Abdullah *et al.*, 2017; Premalatha *et al.*, 2022) .

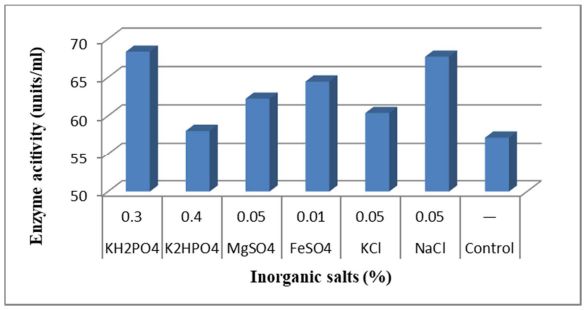

Fig. 8. Effect of inorganic salts on amylase production

Effect of different inorganic salts

Various inorganic salts were incorporated in the production medium at different concentrations to examine the effect on amylase synthesis by *Penicillium* sp. (Fig. 8). The salts examined were KH_2PO_4, K_2HPO_4, $MgSO_4$, $FeSO_4$, KCl and NaCl. The maximum amount of amylase activity was resulted against KH_2PO_4 **(**68.35 units/ml), $FeSO_4$ **(**64.46 units/ml) and NaCl (67.70 units/ml). From various studies it is evident that different inorganic salts are essential for optimum production of various fermented products like enzymes (Sivaramakrishnan *et al.*, 2006; Abdullah *et al.*, 2017; Balakrishnan *et al.*, 2021; Premalatha *et al.*, 2022). The presence of salts may also help in the maintenance of pH and buffering activity of the production medium.

Conclusion

α-amylase is one of the most important enzymes having highest range of applications in various industries which alone share more than 30% of the enzyme market with an increasing demand day by day. Thus the search for new and novel amylase producing microorganism is very much relevant in the present context. In the study optimum production was found at initial pH - 7.0**,** incubation temperature- 30°C, carbon source - Starch (1.5%), organic nitrogen source - Peptone (0.6%), inorganic nitrogen source - $(NH_4)_2SO_4$ (0.02%) and inorganic salt - KH_2PO_4 (0.3%) respectively. The findings suggested that the medium's composition has a significant role in controlling the production of extracellular enzymes. The study indicated that the mold *Penicillium* sp. showing 55 hours of optimum incubation time has the potentiality to be an economically viable organism for commercial production of α-amylases.

References

Abdullah R, Nadeem S, Iqtedar M, Kaleem A, Iftikhar T, Naz S. 2017. Influence of growth conditions on enhanced production of alpha amylase from *Penicillium* species in solid-state fermentation. Indian Journal of Biotechnology **16**(3), 426–432.

Ayansina ADV, Adelaja AO, Mohammed SSD. 2017. Characterization of amylase from some *Aspergillus* and *Bacillus* species associated with cassava waste peels. Advances in Microbiology **7**(4), 280–292. https://doi.org/10.4236/aim.2017.74023.

Balakrishnan M, Jeevarathinam G, Kumar SKS, Muniraj I, Uthandi S. 2021. Optimization and scale-up of α-amylase production by *Aspergillus oryzae* using solid-state fermentation of edible oil cakes. BMC Biotechnology **21**, 33. https://doi.org/10.1186/s12896-021-00686-7.

Binod P, Palkhiwala P, Gaikaiwari R, Nampoothiri KM, Duggal A, Dey K, Pandey A. 2013. Industrial enzymes - present status and future perspectives for India. Journal of Scientific & Industrial Research **72**, 271–286.

Das S, Singh S, Sharma V, Soni ML. 2011. Biotechnological applications of industrially important amylase enzyme. International Journal of Pharma and Bio Sciences **2**, 486–496.

De Souza PM, Magalhaes PO. 2010. Application of microbial α-amylases in industry: a review. Brazilian Journal of Microbiology **41**, 850–861.

Erdal S, Taskin M. 2010. Production of α-amylase by *Penicillium expansum* MT-1 in solid-state fermentation using waste loquat (*Eriobotrya japonica* Lindley) kernels as substrate. Romanian Biotechnological Letters **15**, 5342–5350.

Far BE, Ahmadi Y, Khosroshahi AY, Dilmaghani A. 2020. Microbial alpha-amylase production: progress, challenges, and perspectives. Advanced Pharmaceutical Bulletin **10**(3), 350. https://doi.org/10.34172/apb.2020.043.

Grandview Research. 2024. Alpha-Amylase baking enzyme market size, share & trends analysis report by source (fungi, plant-based), by application (bread, cookies & biscuits), by region (North America, Europe), and segment forecasts, 2024–2030. https://www.grandviewresearch.com/industry-analysis/alpha-amylase-baking-enzyme-market (accessed 2 October 2024).

Gupta R, Gigras P, Mohapatra H, Goswami VK, Chauhan B. 2003. Microbial α-amylases: a biotechnological perspective. Process Biochemistry **38**, 1599–1616.

Miller GL. 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Journal of Analytical Chemistry **31**, 426–428. https://doi.org/10.1021/ac60147a030.

Mostafa FA, Wehaidy HR, El-Hennawi HM, Mahmoud SA, Sharaf S, Saleh SA. 2024. Statistical optimization of α-amylase production from novel local isolated *Bacillus* spp. NRC1 and its textile applications. Catalysis Letters **154**, 3264–3275. https://doi.org/10.1007/s10562-023-04545-2.

Okpara MO. 2022. Microbial enzymes and their applications in the food industry: a mini-review. Advances in Enzyme Research **10**(1), 23–47. https://doi.org/10.4236/aer.2022.101002.

Premalatha A, Vijayalakshmi K, Shanmugavel M, Rajakumar GS. 2023. Optimization of culture conditions for enhanced production of extracellular α-amylase using solid-state and submerged fermentation from *Aspergillus tamarii* MTCC5152. Biotechnology and Applied Biochemistry **70**(2), 835–845. https://doi.org/10.1002/bab.2403.

Sindhu R, Suprabha GN, Shashidhar S. 2009. Optimization of process parameters for the production of α-amylase from *Penicillium janthinellum* (NCIM 4960) under solid-state fermentation. African Journal of Microbiology Research **3**(9), 498–503.

Sindhu R, Suprabha GN, Shashidhar S. 2011. Purification and characterization of α-amylase from *Penicillium janthinellum* (NCIM 4960) and its application in the detergent industry. Biotechnol Bioinf Bioeng **1**(1), 25–32.

Singh R, Kim SW, Kumari A, Mehta PK. 2022. An overview of microbial α-amylase and recent biotechnological developments. Current Biotechnology **11**(1), 11–26. https://doi.org/10.2174/2211550111666220328141044.

Sivaramakrishnan S, Gangadharan D, Nampoothiri KM, Soccol CR, Pandey A. 2006. Alpha amylases from microbial sources: an overview on recent developments. Food Technology and Biotechnology **44**, 173–184.

Tiwari KL, Jadhav SK, Fatima A. 2007. Culture conditions for the production of thermostable amylase by *Penicillium rugulosum*. Global Journal of Biotechnology and Biochemistry **2**, 21–24.

Van der Maarel MJEC, van der Veen B, Uitdehaag JCM, Leemhuis H, Dijkhuizen L. 2002. Properties and applications of starch-converting enzymes of the α-amylase family. Journal of Biotechnology **94**, 137–155.

Verified Market Reports. 2024. Global alpha-amylase market by type (plants, bacteria), by application (fruit ripening, medical diagnostics), by geographic scope and forecast. https://www.verifiedmarketreports.com/product/alpha-amylase-market/ (accessed 2 October 2024).

Vidya B, Gomathi D, Kalaiselvi M, Ravikumar G, Uma C. 2012. Production and optimization of amylase from *Penicillium chrysogenum* under submerged fermentation. World Journal of Pharmaceutical Research **1**(4), 1116–1125.