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OPTIMIZATION OF L-ASPARAGINASE PRODUCED BY *OSCILLATORIA TEREBRIFORMI* ISOLATED FROM MEDITERRANEAN SEA COAST, EGYPT

***Reham G. Elkomy**

Marine Biotechnology and Natural Product Extract Laboratory, National Institute of Oceanography and Fisheries, Alexandria, Egypt.

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***Corresponding Author**
***Reham G. Elkomy**
Marine Biotechnology and Natural Product Extract Laboratory, National Institute of Oceanography and Fisheries, Alexandria, Egypt.

ABSTRACT

In this experiment cyanobacterium *Oscillatoria Terebriformis* was isolated from Eastern harbor Alexandria in Mediterranean coast of Egypt. The highest enzyme productivity was observed on the 12th days and the optimized parameters were pH 7, 30^{o}C and 3000 lux. Immobilization using adsorbed on Luffa pulp enhanced the enzyme production compared to the conventional free- cells repeated reuse of the adsorbed cells achieved the maximum enzyme specific activity (55.562U/ml).

KEYWORD: Marine cyanobacterium; optimization; asparaginase; production.

INTRODUCTION

L-asparagine is an essential amino acid used by both normal cells and cancer cells. L-asparaginase enzyme cleaves L- asparagine into aspartic acid and ammonia. Many types of cancerous cells require L-asparagine for protein synthesis they are deprived of an essential growth factor in the presence of L- asparaginase. This enzyme is mainly used in the treatment of acute lymphatic leukemia (ALL) in children.[1,2] These tumor cells require large amount of L- asparagine for malignant growth.[3] L-asparaginase was produced by wide range of algae, bacteria, actinomycetes, fungi, and plants.[4] All the forms of the enzyme have similar functionality and received important attention.[5,6] Recently, L-asparaginase is used in food technology as a potent mitigating agent for reducing the acrylamide (AA, CH2=CH-CO-NH2), a potential carcinogen, which is formed in the reaction of L-asparagine) and reducing sugars contained in foods during heating processes.[7] More recently, production of L-

asparaginase from blue green microalgae are receiving more attention due to its high nutrient contents, low cost of production, and cost-effectiveness, no seasonal variation, high efficient producers, being easily cultured and harvested at large scales, and cheaper and easier extraction, and higher yields and purification of protein and enzymes by simple methods are available. However, few specific reports regarding production of L-asparaginase by blue-green algae are recorded.[8,9] Cell immobilization is considered a promising approach for enhancing the fermentation processes, enzymes production and bioremediation of toxic substances.[10,11] Present investigation deals with isolation of l-asparaginase producing marine cyanobacterium *Oscillatoria Terebriformi* as a novel source of enzymes in addition to optimization of different factors as temperature, pH and light intensities for L-asparaginas production plus using different immobilizatio techniques.

MATERIALS AND METHODS

Microalgae isolation

The algal strain was isolated from Eastern harbor Alexandria in Mediterranean coast of Egypt. Sample was grown in F/2 medium.[12,13] Harvesting took place by centrifugation at 5000 rpm for 15 min. The isolated strain was identified according to available literature.[14,15,16]

Preparation of cell-free extracts

The algal growth was separated by centrifugation at 5000 rpm for supernatant was used as crude enzyme source to determine the enzyme activity.

Estimation of L-asparaginase production

L-asparaginase activity was carried out by using Mashburn and Wrist on determination method.[17] The reaction mixture containing 0.5 ml of 0.04 M L-asparagine, 0.5 ml of 0.5 M HCL buffer (pH 7.8), 0.5 ml of an enzyme and 0.5 ml distilled water was added to make up the total volume to 2 ml. The tubes were incubated at 30°C for 30 minutes. The reaction was stopped by adding 0.5 ml of 1.5 M Trichloroacetic acid (TCA). The blank was prepared by adding enzyme after the addition of TCA. To the 3.7 ml of distilled water and 0.2 ml of Nessler's reagent, 0.1 ml from the above mixture was added. After incubating the mixture at 20°C for 20 minutes the OD was checked at 450 nm with Spectrophotometer. The enzyme activity was expressed in International unit.

Optimization of parameter for higher production of L- asparaginase

The asparaginase production of potent isolate was optimized under shake flask culture. The effects of different parameters including temperature, pH and light intensities on enzyme production were studied and asparaginase activity analyzed by standard asparaginase assay.

Temperature optimization

Enzyme activity was detected at different temperatures (20, 25, 30, 35, 40 and 45°C) by using modified F/2 medium and flasks were kept with shaking180 rpm.

pH optimization

Enzyme activity was detected at different pH ranges from 6 to 10, by using modified F/2 medium and flasks were incubated at 30 ºC with shaking at 180 rpm.

Light intensities optimization

Enzyme activity was detected at different light intensities (1000, 2000, 3000 Lux), by using modified F/2 medium and flasks were kept at 30oc and pH 7 under incubation period with shaking at 180 rpm.

Immobilization by adsorption

Two ml of algal growth was added in Erlenmeyer flasks (250 ml capacity) containing 50 ml sterilized culture medium and support materials such as pumice, coal, ceramic, luffa pulp and sponge cubes. The flasks were incubated under static condition at 30 °C, pH7 and 3000 lux for 12 days.[18]

Statistical Analysis

All measurements were carried out in triplicate. Statistical analyses were performed using one-way analysis of variance (ANOVA), and the significance of the difference between means was determined by Duncan's multiple range tests. Differences at $P < 0.05$ were considered statistically significant. The results were presented as mean values (± standard deviations).

RESULTS AND DISCUSION

Microalgae isolated

The algal strain was identified as *Oscillatoria Terebriformis*. The growth of *O. Terebriform is* increased and reached to maximum value at stationary phase after 12th days under 30±2°C, pH 7 and 3000 lux, then, phase started to decrease (Figure 1). in this respect, Becker[19]

concluded that, the most studies on the biochemical production of algal and their analysis were carried out in stationary phase pf growth period.

Microalgae isolation and enzyme production

The algal strain was identified as *O. Terebriformis*. The maximum enzyme production by *O. Terebriformis* was obtained after 12th days (Figure 2) of incubation time (50.150 U /ml) at 30±2°C, pH 7 and 3000 lux. Ellaiah[20] have reported that growth rate and enzyme synthesis of the culture are the two main characteristics which are mainly influenced by incubation time.

Optimization of parameter for higher production of L- asparaginase

Temperature optimization

In the present study, incubation temperature influenced the production of L- asparaginase by *O. Terebriformis.* Manna[21] have reported 37°C as the optimal temperature for maximum activity by *Pseudomonas stutzeri*. In the present study, maximal L- asparaginase observed at 30°C (50.150 U/ml) and minimum production was at 45°C (33.223 U/ml) Figure 3. Further increase in temperatures adversely affected the enzyme production. Sarqius[22] also reported 30°C is suitable for L-asparaginase production through submerged fermentation by using *Aspergillus terreus* and *Aspergillus tamari.* The optimum temperature 30 0r 37 was reported in most of the L- asparaginase production.[23,24]

pH optimization

The pH plays avital role in enhancement of L-asparaginase production. Maximal enzyme production was observed at pH 7 (50.150 U/ml) and pH above and below pH 7 led to a decrease in L- asparaginase production. The minimum enzyme production was observed at pH 10 (32.213 U/ml) Figure 4. Similar results have been reported by De-angeli[25,26] and showed that pH 7.0 is the optimum pH for L-asparaginase production under submerged fermentation process.

Light intensities optimization

Light is an essential key for growth of microalgae. Carvalho[27] have reported that microalgae use light to process the photosynthetic, through the photosynthetic process for autotrophic microalgae to convert carbon dioxide in the air into organic compound, visible light is the main source of energy. The obtained results suggested that the highest production for enzyme was recorded at 3000 lux (50.150 U/ml) Figure 5.

Production of L-asparaginase by *Oscillatoria Terebriformis* adsorbed on different solid supports

Adsorption of *O. Terebriformis* on different solid porous supports such as pumice, coal, ceramic, luffa pulp and sponge cubes for L-asparaginase production was investigated. The results graphically illustrated in Figure 6 showed a significant adsorption of algal cells on luffa pulp, pumice and ceramic supports in terms of enzyme production. Particularly, luffa pulp showed a slightly higher algal adsorption when compared to the other supports. Cultures containing adsorbed algal cells on luffa pulp, pumice and ceramic showed a relatively high L-asparaginase production (55.562, 45.0938 and 43.875 U/ml respectively), while sponge showed the lowest activity (29.906 U/ml). Thus, cultures containing luffa pulp had higher l-asparaginase specific activity (55.562 U/ml) than that of free cultures. Therefore, luffa pulp was selected for production of L-asparaginase. Vijay and Jaya[28] reported that The polyurethane foam as a support material for immobilization of Streptomyces gulbargensis mu24 and L-asparaginase yield was increased by 30.2% as compared to free cells.

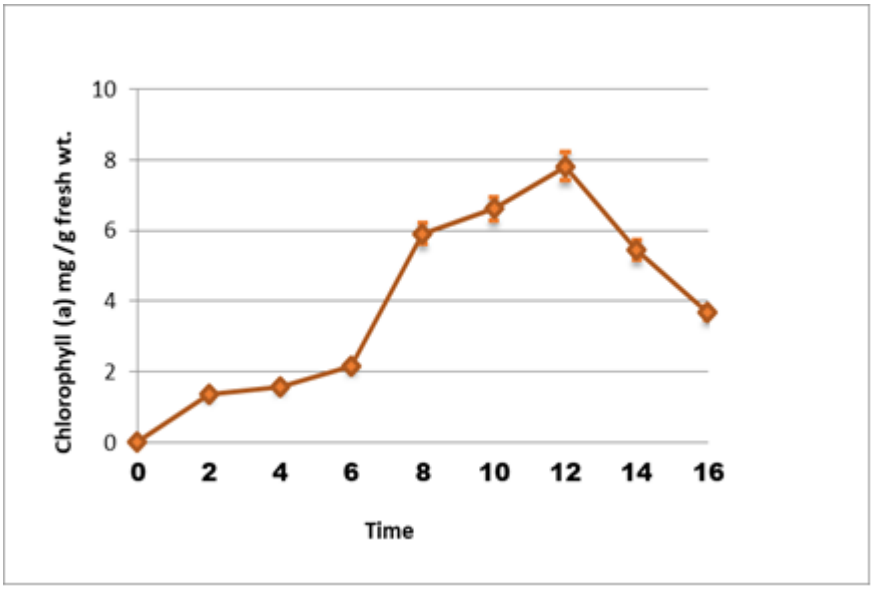

Fig.1: Growth curve of marine cyanopacterium *O. Terebriformis* measured as chlorophyll (a) mg/g fresh wt. Values are the mean of three replicates.

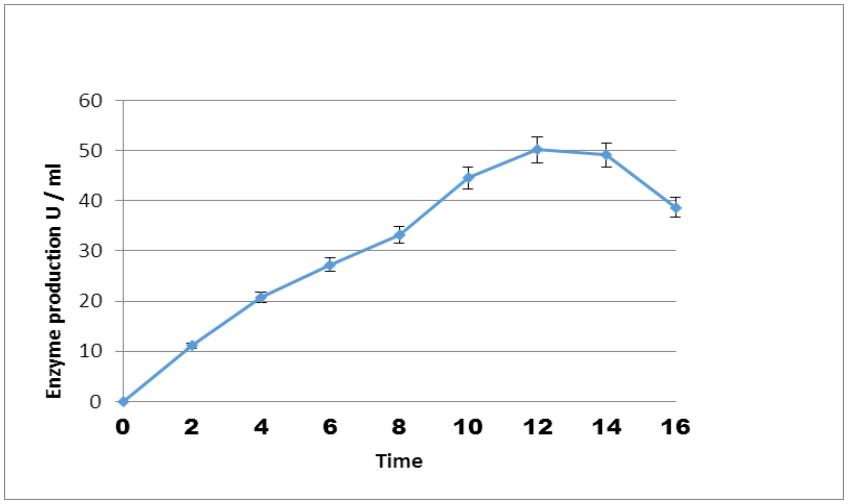

Fig. 2: Effect of incubation time on production of asparginase.

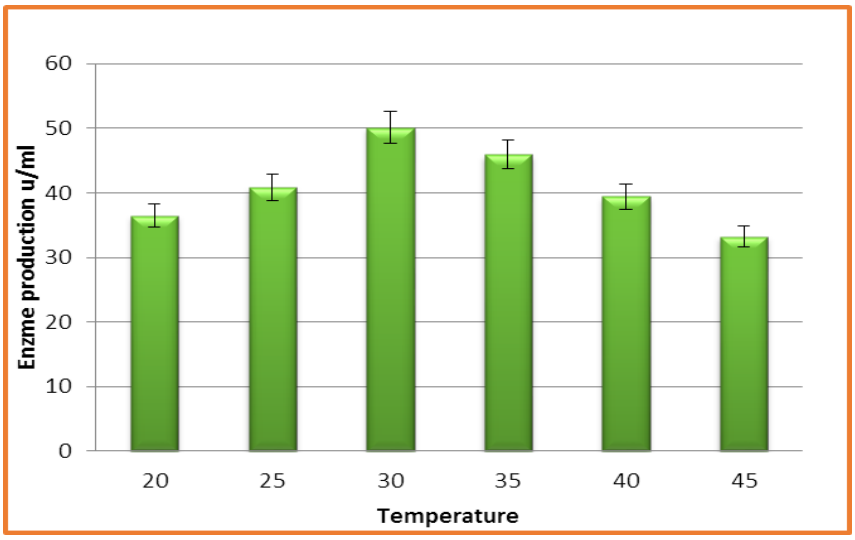

Fig 3: Effect of different temperature on production of asparginase.

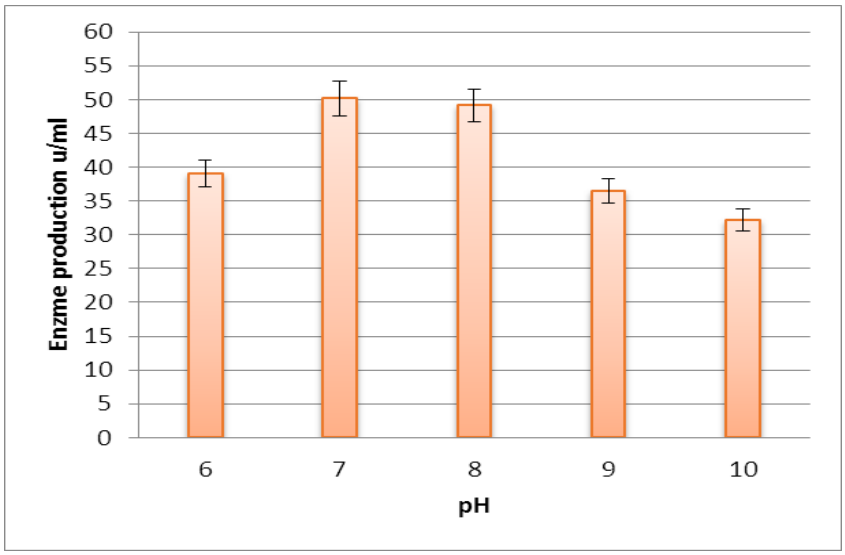

Fig 4: Effect different pH on production of asparginase.

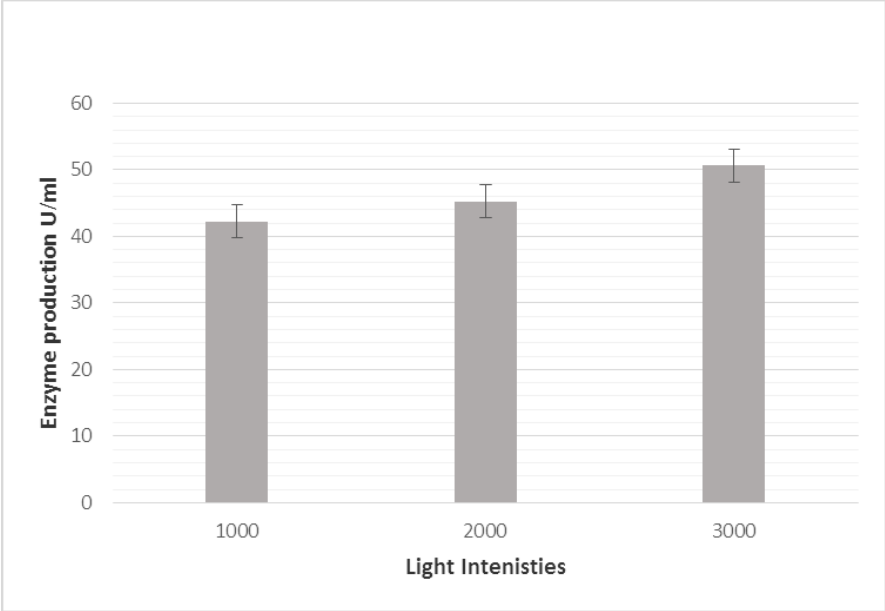

Fig 5: Effect different light intenisties on production of asparginase.

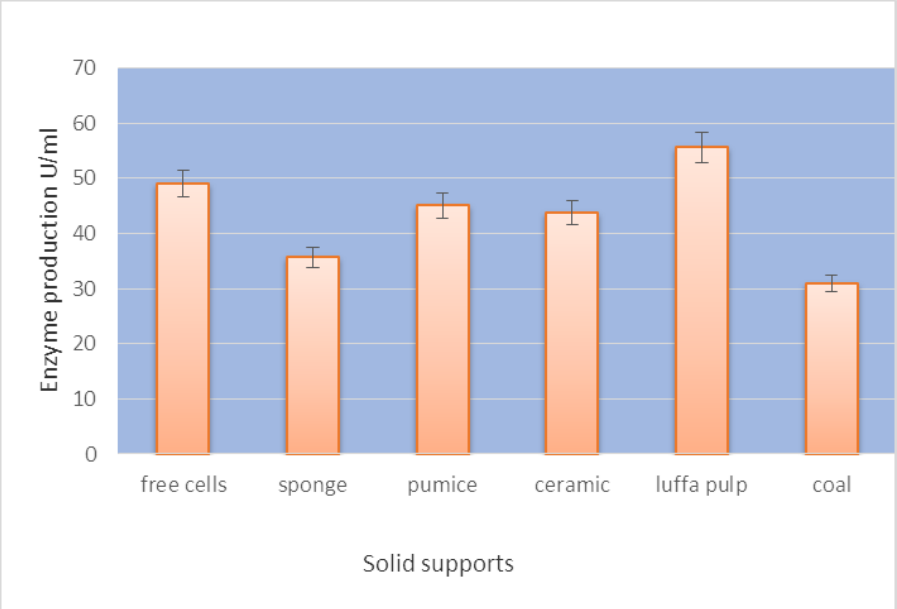

Fig. 6: Production of asparaginase on different solid support.

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CONCLUSION

From present study, it is showed that cyanobacterium *Oscillatoria Terebriformis* can provide a rich source of L-asparaginase producing. Further this study reveals that maximum

production of L-asparaginase enzyme after optimization parameters such as temperature, pH and light intents. Hence, *O. Terebriformis* appears to produce high amount of L-asparaginase enzyme which may be useful in many applications.

REFERENCES

1. Gallagher MP, Marshall RD, Wilson R. Asparaginase as a drug for treatment of acute lymphoblastic leukaemia. Ess Biochem, 1999; 24: 1-40.
2. Verma N, Kumar K, Kaur G, Anand S. L-asparaginase: a promising chemotherapeutic agent. Crit Rev Biotech, 2007; 27: 45-62.
3. Pedreschi F, Kaack K, Granby K. The effect of asparaginase on acrylamide formation in French fries. Food Chem, 2008; 109: 386-392.
4. Sarquis MIM, Oliveira EMM, Santos AS, Costa GL. Production of L-asparaginase by filamentous fungi. Memórias do Instituto Oswaldo Cruz, 2004; 99: 489- 492.
5. Mahajan RV, Saran S, Kameswaran K, Kumar V, Saxena RK. "Efficient production of l-asparaginase from *Bacillus licheniformis* with low-glutaminase activity: optimization, scale up and acrylamide degradation studies," *Bioresource Technology*, 2012, 125: 11–16.
6. Kalra S, Midha A, Kaur A. "Production, purification and characterization of L-asparaginase from *Pseudomonas aeruginosa* 2488 using fermentation," *International Journal of Advanced Research*, 2016; 4: 541–549.
7. Bongers ML, Hogervorst JGF, Schouten L J, Goldbohm RA, Schouten HC, van den Brandt PA., "Dietary acrylamide intake and the risk of lymphatic malignancies: The Netherlands Cohort Study on Diet and Cancer," *PLoS ONE*, 2012; 7: article e38016.
8. Prihanto, Wakayama M. "Combination of environmental stress and localization of l asparaginase in *Arthrospira platensis* for production improvement," *3 Biotech*, 2014; 4: 647–653.
9. Hanaa H, Abd El Baky, Gamal S. "Optmization of growth condition for purification and production of L- Asparaginase by spirulina maxima, Hindawi, 2016; 7: article ID 1785938.
10. Beshay U. Production of alkaline protease by Teredinobacterturnirae cells immobilized in Ca-alginate beads Afr. J. Biotechnol., 2003; 2: 13-60.
11. Kar S, Ray R.C. Statistical optimization of α-amylase production by Streptomyces erumpens MTCC 7317 cells in calcium alginate beads using response surface methodology Pol. J. Microbiol, 2008; 57: 14-49.

12. Guillard RRL, Ryther, JH. Studies of marine planktonic diatoms. I. Cyclotella nana Hustedt and Detonulaconfervacea Cleve. *Can. J. Microbiol.*, 1962; 8: 229–39.
13. Guillard RRL. Culture of phytoplankton for feeding marine invertebrates. In: Smith, W. L., and Chanley, M. H., eds. Culture of Marine Invertebrate Animals. Plenum Press, New York, 1975; 26–60.
14. Tomas C, Hasle GR, Syvertse EE, Steidinger K, Tangen K. Identifying Marine Diatoms and Dinoflagellates. Elsevier, 1996; 598.
15. Prescott GW. The Algae: A review. Boston, Mass.: Houghton Mifflin Co. Pulz O and Gross W. Valuable products from biotechnology of microalgae. *Appl. Microbiol. Biotechnol.;* 1968; 65: 635-648.
16. Cronberg G, Annadotter H. Manual on Aquatic cyanobacteria.ISSHA, 2006;106.
17. Imada A, Igarasi S, Nakahama K, Isona M. Asparaginase and glutaminase activities of microorganisms. J Gen App Microb, 1973; 76: 85-99.
18. Vassilev NB, Vassilev MC, Spassova DI. Production of gluconic acid by *Aspergillus niger* immobilized in polyurethane foam Appl. Microbiol. Biotechnol., 1939; 39: 285-288.
19. Becker Ew. Microalgae biotechnology and microbiology. Cambridge University Press, Cambridge; 1994.
20. Ellaiah P, Srinivasulu B, Adinarayana K. A review on microbial alkaline proteases. J Scient Indus Res, 2002; 61: 690-704.
21. Manna S, Sinha A, Sadhukan R, Chakrabarty SL. Purification, characterization and antitumor activity of L-asparaginase isolated from *Pseudomonas stutzeri* MB-405. Curr Microb, 1995; 30: 291-298.
22. Sarquis MIM, Oliveira EMM, Santos AS, Costa GL. Production of L-asparaginase by filamentous fungi. Memórias do Instituto Oswaldo Cruz, 2004; 99: 489- 492.
23. Lapmakm K, Lumyong S, Thongkuntha S, Wongputtisin P, Sarsud U. "L- asparaginase production by Bipolaris sp. Br438 isolated from brown rice in L- asparaginase producing microorganisms" let. Appl. Microbiol. 2010; 24: 23-26
24. Hosamamani R, Kaliwal BB. "Isolation, Identification and Optimization of fermentation parameters for the production of L-asparaginase, an anticancer agent by *fusarium equiseti*" int. j. microbial; 2011; res 3: 108-119.
25. De-Angeli C, Pocciari F, Russi S, Tonolo A, Zurita VE, Ciaranf E, Perin A. Effect of L-asparaginase from *Aspergillus terreus* on ascites sarcoma in the rat. Nature, 1970; 225: 549-550.

26. Monica T, Lynette L, Francois NN, Sunil SM. “Isolation purification and characterization of fungal extracellular L-asparaginase from Mucor Hiemalis, j. Biocatal., Biotrans. 2014; 2, 2.
27. Carvallho PA, Silva OS, Baptista MJo, Malcata FX. Light requirements in microalgae photobiorectors: an overview of biphotonic aspects. Appl Microbiol Biotechnol, 2011; 89 p. 1275-1288.
28. Vijay B, Jaya Raju K. “Productio of L-asparaginase by aspergillus terreus MTCC 1782 under solid state fermentation using pearl millet and finger milte as mixed substrate” j. Chem. Biol. Phys. Sci. 2015; B 5(1): 366-377.