International Journal of Health Technology and Innovation
An Official Journal of **Kalam Institute of Health Technology** | ISSN - **2583-8547**
RESEARCH ARTICLE

Optimization of Bio-parameters of Fermentation Medium for L-Glutaminase Production by using *Aspergillus flavus* Strain S4

Vinjarapu Hemalatha[1], Sruthi Saladula[2], Kolla Venkata Atchuta Rao[2], Kalyani Pullapukuri[1*]

[1]*Department of Microbiology, Andhra University, Visakhapatnam-530003 India.*
[2]*I Hub building, AMTZ Campus, Pragati Maidan, Visakhapatnam-530031 India.*

Received: 23rd January, 2025; Revised: 21st February, 2025; Accepted: 23rd March, 2025; Available Online: 01st April, 2025

ABSTRACT

Due to its potential for treating lymphoblastic leukemia, the amidohydrolase enzyme L-glutaminase has attracted a lot of attention. Using a variety of agro-industrial residues, this study aims to maximize the extracellular synthesis of L-glutaminase by *Aspergillus flavus* strain S4. To identify ideal production conditions, the effects of important process variables were assessed, such as substrate type, incubation time, temperature, moisture content, starting pH, additional carbon and nitrogen sources, and metal ions. On the second day of incubation, at pH 6.0, 30°C, and 50% moisture content, *A. flavus* S4 was cultivated on tea dust and produced the most glutaminase under ideal conditions using solid-state fermentation (SSF). The addition of sodium nitrite and dextrose as dietary elements improved production even further.

Keywords: L-glutaminase, *Aspergills flavus*, Solid state fermentation

International Journal of Health Technology and Innovation (2025)

How to cite this article: Hemalatha V, Saladula S, Rao KVA, Pullapukuri K. Optimization of Bio-parameters of Fermentation Medium for L-Glutaminase Production by using Aspergillus flavus Strain S4. International Journal of Health Technology and Innovation. 2025;4(1):58-63.

Doi: 10.60142/ijhti.v4i01.09

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

The hydrolytic enzyme L-glutaminase (also known as L-glutamine amidohydrolase, E.C. 3.5.1.2) catalyzes the transformation of L-glutamine into L-glutamate and ammonia. Both prokaryotes and eukaryotes rely heavily on glutaminase activity for nitrogen metabolism. Glutaminase has garnered a lot of attention lately due to its numerous uses in the food industry as a flavor and aroma enhancer and in pharmaceuticals as an anticancer agent (Roberts *et al.,* 1970, 1976) and an effective anti-retroviral agent (Roberts and McGregor, 1991). Another significant use for glutaminase is as a biosensor to track glutamine levels in hybridoma and mammalian cell cultures without requiring a separate glutamic acid assay (Sabu *et al.,* 2000).

Numerous microorganisms, including bacteria, fungi, and yeast, as well as animal tissues and plants, contain L-glutaminase 6–8, with fungi being the most powerful producers (Balagurunathan *et al.,* 2010). *Aspergillus* and *Trichoderma* are the primary industrial producers of glutaminases (El-Sayed *et al.,* 2009). In the upcoming decades, the best source of chemicals found in nature will be the marine endophytic microflora. Effective therapeutic enzymes can be obtained from natural compounds metabolized by endophytic microbes (Weng *et al.,* 2009; Amina *et al.,* 2009). The discovery of new cancer treatments has been aided by the abundance of physiologically active compounds found in marine fungi, particularly those belonging to the genera *Penicillium*, *Aspergillus*, and *Fusarium*. Despite receiving less research attention than their terrestrial counterparts and other ecological groups, marine fungi must be researched since they produce novel compounds that are not present in terrestrial fungi.

Numerous researchers have reported that microbial enzyme output may be increased by optimizing the growing medium's physical and chemical parameters (Mahalakshmi *et al.,* 2009). According to Kashyap *et al.* (2002), the majority of microorganisms, including bacteria, yeast, molds, and filamentous fungi, are known to produce L-glutaminase. Numerous terrestrial bacteria, including *E. coli*, *Pseudomonas* sp., *Acinetobacter* sp., *Bacillus* sp., *P. morganni*, *Candida*, and *A. oryzea*, have been shown to possess glutaminlytic activity. Few marine bacteria, such as *P. flurosence*, *Micrococcus luteus*, and *B.bassiana*, are capable of producing L-glutaminase. Fungi are the most powerful producers (Chandrasekaran *et al.,* 1977).

**Author for Correspondence: pkalyani156@gmail.com*

Although L-glutaminase can be derived from plant and animal sources, microbial production is preferred in industrial settings due to its cost-effectiveness, reliability, and ease of regulation and optimization. *Aspergillus* and *Trichoderma* sp. are the primary manufacturers of glutaminases in industry (Tomita *et al.,* 1988). The extensive use of L-glutaminase in numerous fields has always prompted researchers to look for a new and affordable source of the enzyme. This study's goal was to maximize L-glutaminase output by improving fermentation conditions using *Aspergillus flavus***.**

MATERIALS AND METHODS

Influence of of incubation period

Depending on the species, or even the same species isolated from different sources, different incubation times are required to achieve the highest enzyme yield. The fungal culture was placed in the growth media, and the enzyme activity was measured at various incubation times from 24 to 120 hours, while maintaining the other parameters constant, in order to determine the ideal incubation duration for enzyme production. Enzyme activity was measured after samples were aseptically removed every 24 hours.

Influence of of temperature

After preparing the potato dextrose broth (PDB), a fungal culture was added. While maintaining all other parameters constant, the flasks were incubated for two days at various temperatures (20, 25, 30, 35, and 40°C). After two days of incubation, an enzyme assay was used to identify the ideal temperature for maximum enzyme production.

Influence of pH

While maintaining constant values for all other parameters, the impact of pH on the synthesis of selected enzymes was investigated at selected pH levels (2, 3, 4, 5, 6, 7, 8, 9, 10, 11). NaoH/HCl was used to modify the broth's pH in order to make potato dextrose broth. After receiving a fungal culture inoculation, this PDB was cultured for two days. After two days of incubation, the amount of enzyme produced was measured using an enzyme assay.

Influence of Size of inoculum

The inoculum's size has been investigated in order to assess the fungal enzyme yield. By measuring the L-glutaminase activity while holding the other parameters constant, the impact of different inoculum size concentrations—ranging from 1 to 10% of a 24-hour-old active culture with a 0.5% variation—on L-glutaminase synthesis was investigated.

Influence of carbon source

To determine the maximal amount of enzymes produced by the fungal culture, several carbon sources (lactose, fructose, maltose, and sucrose) were added at a rate of 5% in PDB. After two days of incubation, an enzyme assay was performed from each carbon source flask to identify which source produced the most enzymes while maintaining constant other parameters.

Influence of nitrogen source

For increased enzyme production, the need for an extra nitrogen supply was evaluated. Different nitrogen sources (beef extract, potassium nitrate (KNO3), sodium nitrate (NaNO3), soybean, and yeast extract) were added to PDB at a rate of 5% while maintaining the same levels of other parameters. After two days of incubation, the enzyme test was used to determine the maximum amount of enzyme produced.

RESULTS AND DISCUSSION

Influence of incubation period

Depending on the type of enzyme production, the incubation duration has different effects. A brief incubation time presents the possibility of low-cost enzyme manufacturing. As illustrated in Figure 1, when the fermentation medium was incubated at various intervals of 24, 48, 72, 96, and 120 hours, the highest enzyme activity (196 ± 0.6 U/mL) and lowest (89 ± 0.63 U/mL) were detected within 48 and 120 hours, respectively, at 30°C. Durgasi *et al*. (2016) found that *Aspergillus wentii* produced the maximum enzyme production (177.66 U/gds) after 144 hours of incubation. In close agreement with the current investigation, Nathiya *et al.,* 2011 reported a 120-hour incubation duration utilizing *A. fumigatus* in solid-state fermentation.

Influence of inoculum size

It is commonly acknowledged that inoculum size has a significant impact on the microbial fermentation process. The 2% inoculum was determined to be the most appropriate for high L-glutaminase synthesis by *A. flavus* in submerged fermentation at 48 hours of fermentation, out of the five inoculum sizes examined (1, 2, 3, 4, and 5%). At 2% inoculum size, the highest enzyme activity (189.65 U/mL) was recorded, whereas at 5% inoculum size, the lowest enzyme activity (99.11 U/mL) was displayed. According to Nathiya *et al.,* (2011), the 2 mL of a 6-day-old fungal culture was applied as an inoculum, and the highest amount of glutaminase (14.19 U/g of dry substrate) was seen. There was weak glutaminase synthesis at both lower and higher inoculum levels. Providing the ideal inoculum level for fermentation operations is crucial (Pallem *et al.,* 2010). The inoculum concentration ranged from 1.0 to 3.0%, according to

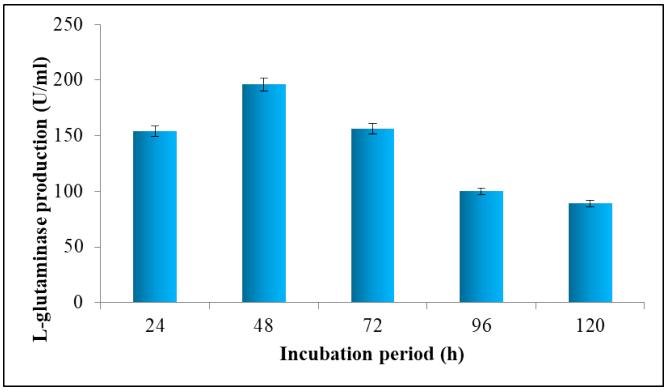

Figure 1: Influence of the incubation period

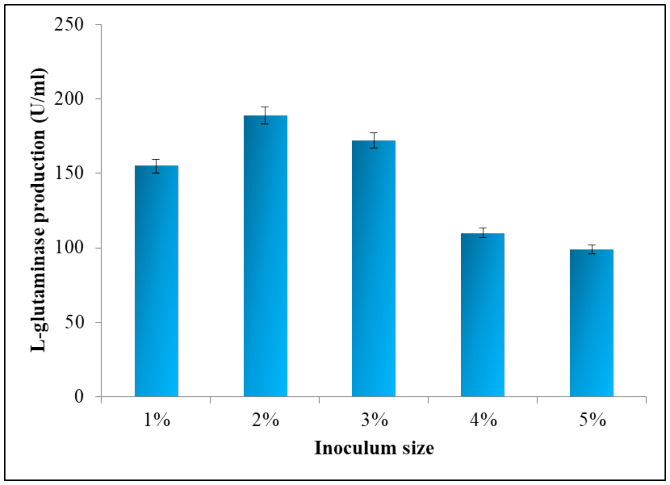

Figure 2: Influence of inoculum size

Sateesh and Prakasham (2012), and the L-glutaminase activity was tracked while the isolated *Bacillus subtilis* RSPGLU was growing. The highest level of production of a selected enzyme (176 U/mL) was recorded at 2.0% of the starting conditions with inoculum supplementation (Figure 2).

Influence of pH

One of the most crucial environmental factors influencing mycelium development, enzyme activity, and the movement of different components across the cell membrane is the medium's pH. The findings demonstrated that the medium's pH of 6 was ideal for promoting the fungal isolate's development and generating a large amount of enzyme. The activity of the enzyme L-glutaminase was actively reduced when the medium's pH was raised or lowered. After two days of incubation, the enzyme L-glutaminase showed its maximum activity at pH 6. At pH 6, the enzyme's activity was 221.53 U/mL. pH was adjusted to preserve the ideal circumstances for higher L-glutaminase synthesis. It was found that the synthesis of L-glutaminase was significantly impacted by pH (Figure 3).

According to Lakshmi Prasanna *et al.,* (2011), the highest synthesis of L-glutaminase 35.39 (U/gds) was achieved at neutral pH (7.0). According to Sunil *et al.,* (2013), the *A. oryzae* S2 strain during submerged fermentation produced the most L-glutaminase at a pH of 5.0. The pH range of 3.5 to 7.0 is known to be optimal for fungal strains, and low pH also prevents contamination by other microorganisms (Pandey *et al.,* 2001). Our results closely match those of Nathiya *et al.,* (2011), who demonstrated that pH 6 was ideal for the highest level of L-glutaminase synthesis. At increasing pH values, L-glutaminase was progressively reduced until it reached 80.25 U/mL at pH 9 and 27°C. On the other side, maximum enzyme production was promoted by incubating *Beauria* sp. and *A. fumigatus* at 27 and 30°C (Sabu *et al.,* 2002). The enzyme biosynthesis was guaranteed in *S. Variabilis* ASU319 and *A. fumigatus* at initial pH values of 4 and 6, respectively (Nathiya *et al.,* 2010). However, Prasanna and Raju (2011) found that, out of a broad range of initial pH and incubation temperatures,

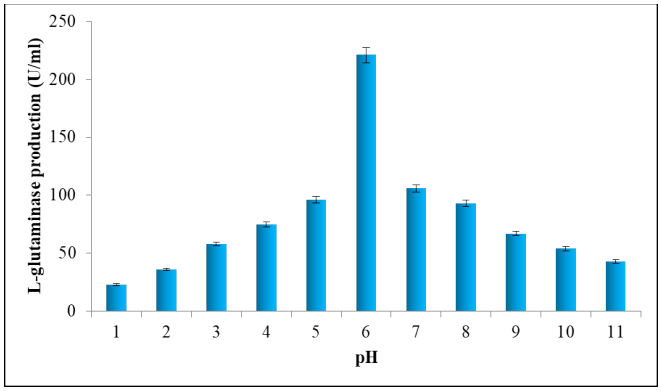

Figure 3: Influence of pH

the highest yield of selected enzyme synthesis was reached at 30°C and pH 7.

Effect of temperature

L-glutaminase synthesis in a number of microbial species has been found to vary with incubation temperature (Sivakumar *et al.,* 2006). In light of this, studies were carried out to determine how temperature affected *A. flavus* S4's ability to produce L-glutaminase. After being injected in PDB media, the organism was cultured for two days at different levels of temperatures (20, 25, 30, 35, and 40°C). After two days of incubation, the enzyme L-glutaminase showed its maximum activity at 30°C. It was discovered that 30°C was the ideal temperature for the enzyme activity. The highest enzyme production of 296.53 U/mL was seen at 30°C out of all the tested temperatures. With an enzyme production of 132.38 U/mL, 45°C was shown to be the lowest enzyme-producing temperature (Figure 4).

The synthesis of the selected enzyme glutaminase by *B. subtilis* was controlled by the temperature. The highest enzyme production of 167 U/mL at 37°C was reported (Satish *et al.,* 2012). Lakshmi Prasanna *et al.,* (2012) conducted fermentation at a range of temperatures, including 26, 28, 30, 32, 34, and 36°C, in order to investigate the impact of temperature on the generation of enzymes. The metabolic activities of microorganisms are shown to be negatively impacted by

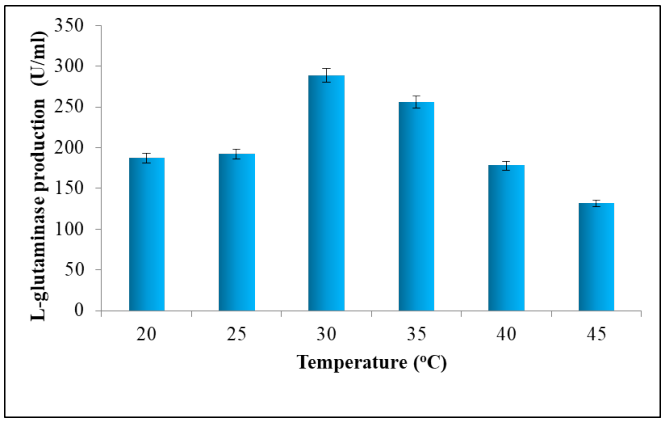

Figure 4: Influence of temperature

any temperature that exceeds the ideal range. Additionally, different scientists have discovered that the metabolic activities of bacteria slow down at lower or higher temperatures (Okolo *et al.,* 1995). The ideal temperature for the highest amount of enzyme synthesis was found to be 30°C. Glutaminase from *A. flavus* (Rashmi *et al.,* 2012) and *Trichoderma koningii* (Chanakya *et al.,* 2010) showed similar results.

Influence of carbon source

The synthesis of cell constituents depends on carbohydrates, which are the most important component of the culture medium. The greatest carbon source among those examined was dextrose, which allowed *A. flavus* strain S4 to produce the most L-glutaminase (376.23 U/mL). With an enzyme synthesis of 198.92 U/mL, soybean meal was shown to be the least enzyme-producing carbon source. Chanakya *et al.,* (2010) investigated the impact of various carbon sources on the synthesis of glutaminase enzyme in *T. koningii*. The best carbon source among the different sugars examined for its impact on L-glutaminase production was maltose, which produced 16.38 U/gds of L-glutaminase. Chitanand *et al.,* (2012) also investigated the effect of various carbon sources on the production of enzymes in *Pseudomonas fluorescens*, they discovered that the preferred carbon source was glucose. Anusri *et al.,* (2014) also investigated the effect of various carbon sources on the synthesis of L-glutaminase in the *Mucor racemosus* strain and they discovered that the preferred carbon source was dextrose. The highest glutaminase production was stimulated by sucrose, followed by glucose and soluble starch from *Aspergillus wentii* MTCC-1901 (Reventh *et al.,* 2013) (Figure 5).

Similarly, Iyer and Singhal (2009) and (2010) found that adding glucose and maltose, respectively, to the production media had a major action on the synthesis of glutaminase by *Providencia* sp. and *Klebsiella oxytoca*. When glucose is added as a carbon source, *Streptomyces* sp. SBU1 (Krishnakumar *et al.,* 2011) and *Penicillium brevicompactum* NRC 829 (Elshafei *et al.,* 2014) produce more L-glutaminase.

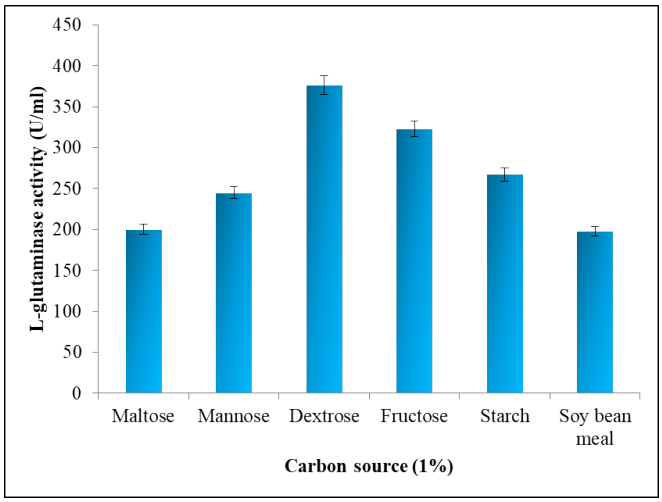

Figure 5: Influence of different carbon sources

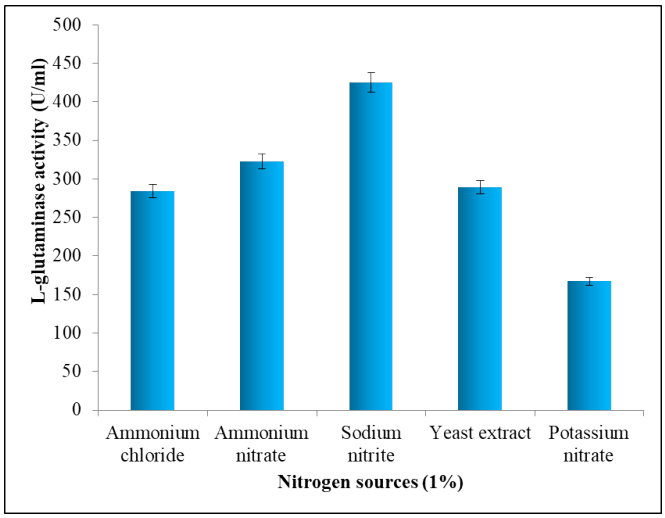

Figure 6: Influence of different nitrogen sources

Influence of nitrogen source

The production of the enzyme was increased by adding more nitrogen sources. The best nitrogen source among those examined was sodium nitrite, which allowed the *A. flavus* strain S4 to produce the highest yield of 425.84 U/mL. With an enzyme production of 167.46 U/mL, potassium nitrate was shown to be the least nitrogen-producing source (Figure 6). Additional organic or inorganic nitrogen sources, such as sodium nitrate, ammonium nitrate, ammonium sulfate, yeast extract, malt extract, urea, and peptone, have been demonstrated to have a significant effect on *A. oryzae's* ability to produce L-glutaminase. Of the different nitrogen sources, the medium's sodium nitrate encouraged 45.19 U/gds of L-glutaminase production and improved microbial growth. These findings were comparable to those published by Prashanth Kumar and colleagues (2009).

CONCLUSION

This work emphasizes *A. flavus* strain S4's potential as a viable microbial source for the synthesis of L-glutaminase. Because of its therapeutic and industrial uses, L-glutaminase has recently attracted a lot of attention from the food and pharmaceutical industries. The study highlights the financial advantages of using affordable agro-industrial wastes as substrates, which provide a feasible and sustainable substitute for submerged fermentation. Solid-state fermentation (SSF) offers a more cost-effective and efficient method that increases enzyme yield while lowering production expenses. The results show that under ideal SSF circumstances, *A. flavus* S4 may generate large amounts of L-glutaminase. This implies that A. flavus S4 has a lot of promise for producing enzymes on an industrial scale. All things considered, this work shows that using microbial fermentation and agro-industrial waste for enzyme biosynthesis is feasible, opening the door for economical and sustainable bioprocesses in the food and pharmaceutical industries.

REFERENCES

1. Roberts J, Holcenberg JS and Dolowy WC (1970). Antineoplastic activity of highly purified bacterial glutaminases. Nature.,

227(5263)1136-1137.
2. Roberts JO (1976). Purification and properties of a highly potent antitumor glutaminase-asparaginase from *Pseudomonas* 7A. Journal of Biological Chemistry., 251(7):2119- 23.
3. Sabu A (2000). L-glutaminase production by marine *Beauveria* sp. under solid state fermentation. Process Biochemistry., 35(7):705-710.
4. Balagurunathan R, Radhakrishnan M and Somasundaram ST (2010). Lglutaminase producing actinomycetes from marine sediments– selective isolation, semi quantitative assay and characterization of potential strain. Australian Journal of Basic and Applied Sciencies., 4(5):698-705.
5. El-Sayed AS (2009). L-glutaminase production by *Trichoderma koningii* under solid-state fermentation. Indian journal of microbiology., 49(3):243-250.
6. Amina N, Hussain MM, Muhammad A, Tassawar H, Butt IF, Khan SA and Ambreen A (2009). Association of response to combined interferon alpha-2b and ribavirin therapy in patients of chronic Hepatitis C with serum alanine aminotransferase levels and severity of the disease on liver biopsy. Journal of Ayub Medical College., 21(2):103-106.
7. Weng CJ, Chau CF, Yen GC, Liao JW, Chen DH and Chen KD (2009). Inhibitory effects of *Ganoderma lucidum* on tumorigenesis and metastasis of human hepatoma cells in cells and animal models. Journal of agricultural and food chemistry., 57(11):5049- 5057.
8. Mahalaxmi Y, Sathish T and Prakasham RS (2009). Development of balanced medium composition for improved rifamycin B production by isolated *Amycolatopsis* sp. RSP- 3. Letters in applied microbiology., 49(5):533-538.
9. Kashyap P, Sabu A, Pandey A, Szakacs G and Soccol CR (2002). Extra-cellular L- glutaminase production by *Zygosaccharomyces rouxii* under solidstate fermentation. Process Biochemistry., 38(3):307-312.
10. Chandrasekaran M (1997). Industrial enzymes from marine microorganisms: The Indian scenario. Journal of Marine Biotechnology., 5(2):86-89.
11. Tomita K, Yano T, Kumagai H and Tochikura T (1988). Formation of γ- glutamylglycyglycine by extracellular glutaminase of *Aspergillus oryzae*. Journal of fermentation technology., 66(3):299-304.
12. Nathiya K, Nath SS, Angayarkanni J and Palaniswamy M (2011). Optimised production of L- glutaminase: A tumour inhibitor from *Aspergillus flavus* cultured on agroindustrial residues. African Journal of Biotechnology., 10(63):13887-13894.
13. Pallem C, Manipati S and Somalanka SR (2010). Process optimization of Lglutaminase production by *Trichoderma koningii* under solid state fermentation (SSF). International Journal of Applied Biology and Pharmaceutical Technology., 1(3):1168-1174.
14. Sathish T and Prakasham RS (2012). Effect of various environmental parameters on secretion of L-glutaminase from *Bacillus subtilis* RSP-GLU. International Journal of Chemistry and Pharmaceutical Sciencies., 1:625-631.
15. Prasanna KL and Raju KJ (2011). Production of L-glutaminase by *Aspergillus oryzae* NCIM 1212 under solid state fermentation using agro – residues. An International Peer Review E-3 Journal of Sciences., 2(1):261-269.
16. Sunil Dutt P, Siddalingeshwara KG, Karthic J, Mushtaq MS and Prathiba KS (2013). An approach on microbial biosynthesis of L-glutaminase: a tumour inhibitor. Journal of Applied Pharmaceutical Science., 3(6):132-135.
17. Pandey A, Soccol CR, Rodriguez-Leon JA and Nigam P (2001). Solid state fermentation in biotechnology. New Delhi: Asia Tech., 237.
18. Sabu A (2002). Sources, properties and applications of microbial therapeutic enzymes. International Journal of Biological Macromolecules., 02(3):334-341.
19. Prasanna KL and Raju KJ (2011). Production of L-glutaminase by *Aspergillus oryzae* NCIM 1212 under solid state fermentation using agro – residues. An International Peer Review E-3 Journal of Sciences., 2(1):261-269.
20. Sivakumar K, Sahu MK, Manivel PR and Kannan L (2006). Optimum conditions for L- glutaminase production by actinomycete strain isolated from estuarine fish, Chanos chanos (Forskal, 1775). Indian Journal of Experimental Biology., 44(3):256-258.
21. Sathish T and Prakasham RS (2012). Effect of various environmental parameters on secretion of L-glutaminase from *Bacillus subtilis* RSP-GLU. International Journal of Chemistry and Pharmaceutical Sciencies.,1:625-631.
22. Lakshmi Prasanna K and Jaya Raju K (2012). Production of L-glutaminase by *Aspergillus oryzae* NCIM 1212 under solid state fermentation using agro-residues. Journal of Chemical, Biochemical and Physical Sciences., 2(1):2249-1929.
23. Okolo BN, Ezeogu LI and Mba CN (1995). Production of raw starch digesting amylase by *Aspergillus niger* grown on native starch sources. Journal of the Science of Food and Agriculture., 69(1):109-115.
24. Rashmi AM, Gopinath SM and Krishan Kumar NMT (2012). Optimization of submerged fermentation process for L-glutaminase produced by *Pseudomonas aeruginosa* BGNAS-5. International Journal of Latest earch in Science and Technology., 1(4):308-310.
25. Chanakya P, Srikant M and Subba Rao S (2010). Process optimization of Lglutaminase by *Trichoderma koningii* under solid state fermentation [SSF]. International Journal of Applied Biology and Pharmaceutical Technology., 1(2):1168-1174.
26. Chitanand MP and Shete HG (2012). Condition optimization and production of extracellular L-glutaminase from *Pseudomonas fluorescens*. International Journal of Pharma and Bio Sciences., 3(3):155-162.
27. Anusri T, Sankar J, Pavani Bai B and Ramachandra Rao CSV (2014). Studies on isolation, screening and molecular charaterisation of L-glutaminase producing marine isolates. International Journal of Science Innovations and Discoveries., 4(1):33-44.
28. Revanth B and Jaya Raju K (2013). L-glutaminase production by *Aspergillus wentii* Mtcc 1901 Under Solid State Fermentation Using Mixed Agro Industrial Residues, International Journal of Chemical Science., 11(1):277-290.
29. Iyer P and Singhal RS (2010). Production of glutaminase (EC 3.2. 1.5) from *Zygosaccharomyces rouxii* in solid-state fermentation and modelling the growth of *Z. rouxii* there in. Journal of microbiology and biotechnology., 20(4):737-748.
30. Iyer PV and Singhal RS (2009). Screening and selection of marine isolate for Lglutaminase production and media optimization using response surface methodology. Applied biochemistry and biotechnology., 159(1):233-250.
31. Krishnakumar S, Rajan RA and Ravikumar S (2011). Extracellular production of L- glutaminase by marine

alkalophilic *Streptomyces* sp., SBU1 isolated from Cape Comorin coast. Indian Journal of Geomarine Sciencies., 40(5):712-721.

32. Elshafei AM, Hassan MM, Ali NH, Abouzeid MAE, Mahmoud DA and Elghonemy DH (2014). Purification, kinetic properties and antitumor activity of L-glutaminase from *Penicillium brevicompactum* NRC 829. British Microbiology Research Journal., 4(1):97-115.
33. Prasanth Kumar K, Prabhakar T, Satish T, Girija Sankar G, Moges F, Swarajya Lakhmi G and Ramana H (2009). Screening of l-glutaminase producing marine bacterial cultures for extracellular production of lglutaminase. International Journal of ChemTech Research., 1(4):1232-1235.