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Review Article

Diverse origins of microbial L-asparaginases and their current miscellaneous applications

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ABSTRACT

L-asparaginase, also known as amidohydrolase, catalyzes the breakdown of asparagine into aspartic acid and ammonia. Due to its ability to inhibit the biosynthesis of protein lymphoblasts, it is used to treat acute lymphoblastic leukemia (ALL). It also has other applications in the food industry by preventing the formation of acrylamide. Different organisms including bacteria, fungi, actinomycetes, and plants produce L-asparaginase. This review highlights different applications of L-asparaginase in the industrial fields, the major sources of L-asparaginase, its immunological reactions and production techniques through the solid state (SSF) and submerged (SmF) fermentation as well as optimization of the production process.

Keywords: *L-asparaginase; Characterization; Optimization; Acute lymphoblastic leukemia.*

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1. INTRODUCTION

Enzymes play an important role in metabolic and biochemical reactions and microorganisms are the primary source **[1]** because they can be cultured in large quantities in a short span of time **[2, 3]**. Proteins that are used as therapeutics have made a great effect in the healthcare area, as they are specific in the disease, highly effective drug, and safe. They have a broad difference of specific utilities as thrombolytics and oncolytic. One of this therapeutic protein is L-asparaginase which has been found to be effective for the treatment of acute lymphoblastic leukemia**[4]**.

Malignant cells differ from the normal cells, that it can barely synthesize L-asparagine, due to the absence of L-asparagine synthetase, so draining the circulating pools of L-asparagine by L-asparaginase leads to tumor cells destruction, so they could not be able to complete the synthesis of the protein through inhibition of RNA and DNA synthesis with subsequent blastic cell apoptosis **[5]**.

L-asparaginase has been combined to the multi-drug chemotherapy in adults and children with Hodgkin's disease, lymphosarcoma, reticulosarcoma, acute lymphoblastic leukemia, acute myelocytic leukemia, and melanosarcoma and it provides significant improvement of the outcomes of the therapy to achieve complete remission in about 90% of patients **[6]**.

Recent advances in food technology have explained that pretreatment of potato slices and bread dough with asparaginase before frying or baking prevents acrylamide formation (carcinogenic toxicant) used in a large number of food products that are prepared at temperatures above 100 ºC **[7]**.

2. L-asparaginase, application, types, and sources

L-asparaginase (asparagine amidohydrolase) is a tetramer protein, otherwise called aminohydrolase relates to the amidase group of the enzyme and can break down L-asparagine into aspartic acid and ammonia **[8]**.

2.1. Its application as a tumor inhibitor

It has a chemotherapeutic characteristic against the tumor cells. It is a powerful curable agent for treatment of lymphosarcoma and acute lymphoblastic leukemia **[9]**. L-asparaginase is the first curative protein with antineoplastic properties that have been examined comprehensively by many researchers far and wide **[8]**.

L-asparaginase is recorded in the nineteenth WHO List of Essential Medicines and WHO Model List of Essential Medicines for Children as a cytotoxic and adjunctive treatment for intense lymphoblastic leukemia. Among different treatments, for example, corticosteroid and vincristine, L-asparaginase is utilized as a remission induction chemotherapy standard treatment alternative for recently diagnosed acute lymphoblastic leukemia **[10]**. It is used also as a central nervous system-directed systemic chemotherapy prophylaxis with dexamethasone and methotrexate for the high and standard-risk acute lymphoblastic leukemia. However, it could not penetrate into the cerebrospinal fluid (CSF), but it could deplete the CSF asparagine.

L-asparaginase discovery and development as an anti-cancer drug started in 1953 when Kidd initially noticed that the lymphomas in mice and rats disappeared after the treatment with the serum of guinea pig. Subsequent investigations demonstrated that it was the enzyme L-asparaginase which was in charge of the tumor relapse **[11]**. Other authors demonstrated that malignantly changed hematopoietic cells are now and again unfit to form adequate asparagine for their own metabolism when there is no dietary supply of this amino acid. The consumption of the systemic pool of asparagine performed by L-asparaginase will then prompt death to the cells **[12]**. Altenbern in 1954 and Broome in 1965 reported the antitumor activity of L-asparaginase in bacteria and yeast, respectively **[8]**. L-asparaginase is present in guinea pigs serum and rodents but is truant in humans. Two isozymes of L-asparaginase, in particular, type I and type II, have been recognized by Ohnuma **[13]**. Both type I and type II asparaginases are portrayed by enzymatic activity for both L-glutamine and L-asparagine. In any case, type II asparaginase shows higher particular action towards L-asparagine. Type II asparaginase definitely demonstrates antitumor activity and is used as chemotherapeutics in ALL **[8]**.

The molecular basis for L-asparaginase therapeutic use stays badly clarified. In mammalian cells, the amino acid response (AAR) pathway is intended to recognize and react to amino acid inadequacy, which upon ending the supply of L-asparagine, enhances the expression

of asparagine synthetase mRNA in the resistant cells. In any case, the expression of asparagine synthetase in susceptible tumor cells is not upregulated may be due to a decrease of the translation initiation, that is realized by the phosphorylation of eukaryotic initiation factors (e.g. eIF-2 a and eIF4e) by a starvation-activated ribosome-associated kinase **[14]**. L-asparaginase treatment causes specific starvation of the susceptible tumor cells. This L-asparagine starvation prompts the prevention of development of rRNA transcription, trailed by the discouragement of the synthesis of asparaginyl tRNA which limits peptide synthesis. L-asparagine consumption is additionally caused by nucleotide biosynthesis slowing and elongation of the S-phase of the cell cycle. L-asparaginase therapy captures the cell cycle of the tumor cell at the G1 phase before DNA degradation. Ammonium ions (one of the by-products of L-asparaginase activity) change the pH by disruption into the cytosol, which stimulates the pathway of the signal transduction associated with substrate phosphorylation and apoptosis **[15]**. Microbial L-asparaginases are marketed for medical applications under the brand names Kidrolase®, Elspar® **[7]**.

Mechanism of action: Both normal and leukemic cells need the amino acid L-asparagine, for their metabolic requirements. Normal cells can form L-asparagine for their development by using transaminase enzyme that changes oxaloacetate into an intermediate aspartate, which after that transfers an amino group from glutamate to oxaloacetate producing α-ketoglutarate and aspartate. At last, in healthy cells, aspartate is changed into asparagine-by-asparagine synthetase. Neoplastic cells do not have the ability to form the asparagine due to the lack of L-asparaginase synthetase, hence are dependent on the external supply of asparagine for their existence and proliferation. Accordingly, providing the tumor cells with L-asparaginase can deplete all circulating asparagine, which leads to cancer cells starvation and death **(Fig. 1 and 2)**. Clinical information published over the most recent two decades states asparaginase is an essential constituent of treatment protocols for ALL. L-asparaginase is predominant among microorganisms and eukaryotes **[16]**.

2.2. L-asparaginase application in food industry

The University of Stockholm and the Swedish Food Authority affirmed the presence of the cancer-causing agent acrylamide in assortments of heated foods ten years ago. A while after the declaration, researchers demonstrated that acrylamide is synthesized from asparagine and reducing sugars during the Maillard reaction **(Fig. 3) [17]**. Since 2002, the food industry has teamed up with scientists worldwide, in order to lessen the amount of acrylamide in cooked foods. Techniques of mitigation can be isolated into three distinct types. Firstly, using starting materials with a low amount of acrylamide precursors can be utilized to decrease the acrylamide in the last product. Secondly, process parameters may be adjusted, in order to lessen the formation of acrylamide. Thirdly, post-process treatment could be utilized to decrease acrylamide **[18]**. However, the third approach is not used widely; an example is the utilization of supercritical CO_2 extraction to decrease the levels of acrylamide in coffee. Almost 80% of the acrylamide was expelled utilizing this strategy **[19]**. Adding other chemicals before or after heating could reduce the amount of the produced acrylamide in a heated product, numerous added substances were utilized, as glycine who can contend with the asparagine to reduce the final acrylamide production, antioxidants and divalent cations as calcium hydroxide all have their side effects (affecting flavor of food) which prevent to be used commercially **[20]**.

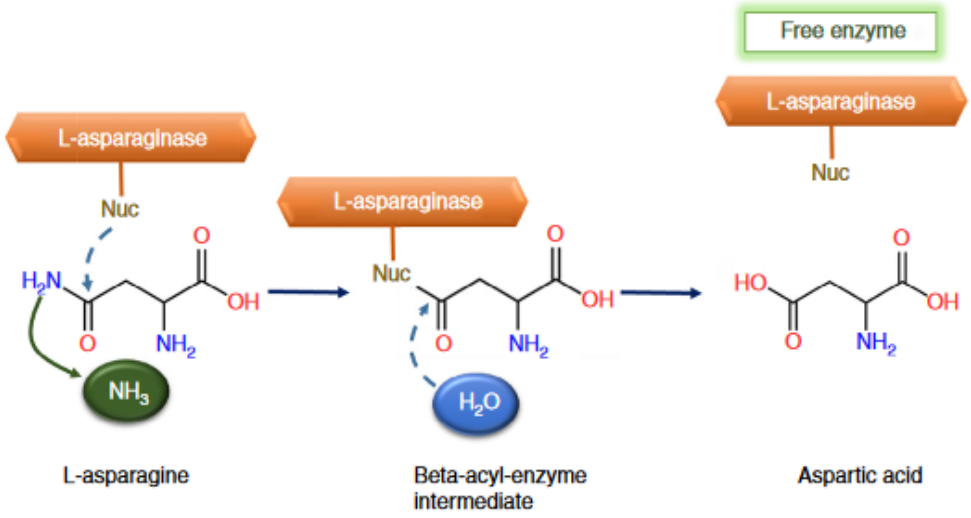

Fig. 1. The general mechanism of L-asparaginase catalyzed reactions **[101]**.

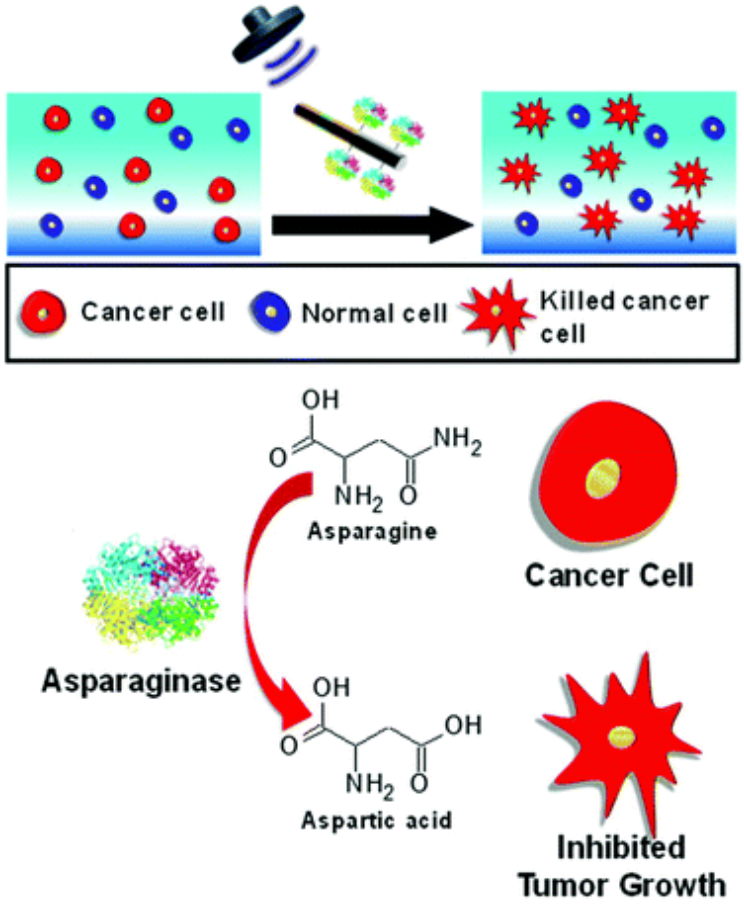

Fig. 2. Mechanism of action of L-asparaginase as an anticancer agent **[102]**.

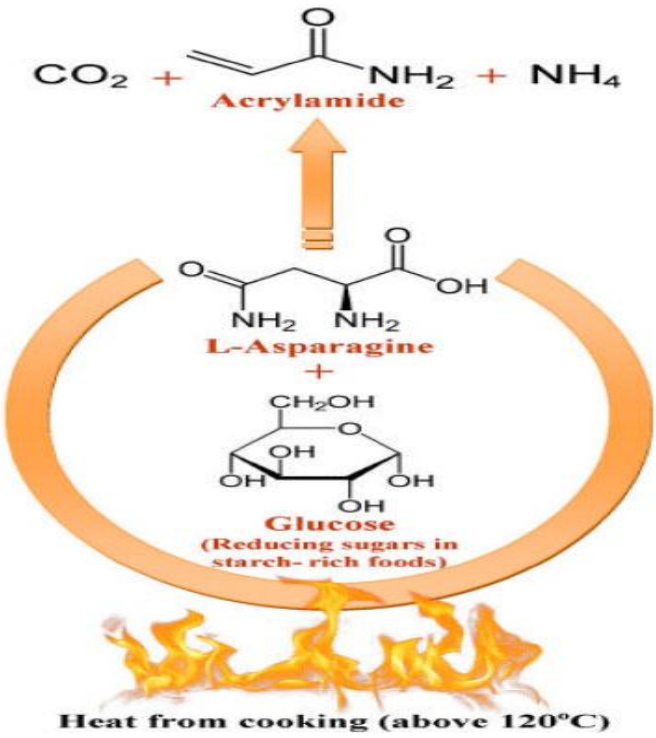

Fig. 3. Schematic illustration of acrylamide **[8]**

Fermentation techniques use certain microorganisms to devour the asparagine or reducing sugar prior to the step of food processing **[21]**. For example, a basal medium including lactic acid bacteria was utilized in the formation of wholemeal rye bread and considerably diminished levels of acrylamide in the final product **[22]**. In addition, of decreasing the pH, the lactic acid bacteria lowered the amount of reducing sugars in the dough.

In the fermentation approach, there are many points which need to be taken into consideration. Starting with, the temperature and pH should be controlled in order to increase the activity of the microorganism to the maximum. Regardless of the reducing sugar consumed was included back after processing, the final product's quality may still be influenced by the fermentation step **[23]**. Besides, fermentation always works in bakery products, with restricted application in the products that are based on potato and also coffee **[24]**.

The use of an enzymatic way to change the reaction pathways was first suggested by Amrein *et al.*, who utilized asparaginase to break down the asparagine to aspartic acid and ammonia **[25]**. This technique is considered to be powerful because asparagine is not a high supporter of the flavor and color of cooked foods at all **[26]**, so required sensory characteristics are kept up. Zyzak *et al*. mentioned that treatment of the potato product with L-asparaginase before cooking might help in 88% reduction of asparagine, as well as acrylamide decrease greater than 99% in the last cooked product **[27]**. Vass *et al*. deduced that at least 70% decrease in the acrylamide level of cracker products can be achieved by the addition of L-asparaginase during the preparation of the dough **[28]**. It is

commercially available under the brand name Acrylaway® and PreventASe® **[7]**.

2.3. L-asparaginase biosensor

Biosensors to recognize asparagine have been made by the capture of L-asparaginase between NH_3 gas permeable membrane and cellophane dialysis membrane **[7]**, which finds deployment as a biosensor. Verma *et al*. performed researches on co-immobilization of L-asparaginase with phenol red indicator on the nitrocellulose membrane, silicone gel and calcium alginate beads, which can be utilized as a biosensor for diagnosis to detect asparagine in the blood samples of normal and leukemia patients by color visualization **[29]**. Verma *et al*. have developed an entire-cell based optic fiber biosensor using L-asparaginase-producing coliform bacteria and phenol red indicator immobilized with each other with Tetramethyl orthosilicate gel, which can discover approach in detecting L-asparagine content in food samples **[30]**.

2.4. Types of L-asparaginase

Bacterial L-asparaginase has been categorized into two large groups named type I and type II based on the homology in the sequence, function, and structure, in addition to various asparaginases that are derived from plant origins **[31]**. Bacterial L-asparaginases belong to a group of amidohydrolases in which threonine is the essential nucleophile during catalysis. Type I L-asparaginases are cytoplasmic and type II are periplasmic, the latter have high affinity to L-asparagine (Km ¼ 10^{-5} M), and to a lesser degree helps in the conversion of L-glutamine into glutamic acid. *E.coli* L-asparaginase type I and II have similar conserved amino acid motifs, but vary in their quaternary structure, and type I is with a much lower affinity towards L-asparagine (Km ¼ 10^{-3} M) **[7]**. Specific strains of yeasts produce L-asparaginases with the same amino acid sequences as the bacterial type II group **[31]**. The amino acid sequences of bacterial L-asparaginases have insignificant homology with plant L-asparaginases.

Plant asparaginases cannot break down glutamine. Most of the plants L-asparaginases are considered N-terminal nucleophile hydrolases that included in metabolic pathways associated with the assimilation of atmospheric nitrogen. Plant L-asparaginases have two types: potassium-dependent and potassium independent, both with prominent levels of sequence similarity to each other. The potassium-dependent enzyme is more broadly disseminated in higher plants and it can effectively metabolize L-asparagine during high metabolic need for nitrogen **[32]**. Yeast and bacterial L-asparaginases are considered type II L-asparaginase group. In spite of the fact that actinomycelial and fungal L-asparaginases are considered as microbial L-asparaginases, their alliance to the current types of L-asparaginases is not well defined **[7]**.

2.5. Sources of L-asparaginases

L-asparaginase presents in different organisms, including animals, plants, and microorganisms (bacteria, fungi, algae, yeast, and actinomycetes) except humans. Even though L-asparaginase presents in various animal and plant groups, but because of its difficult extraction procedures, other conceivable sources (bacteria, fungi, algae, yeasts, and actinomycetes) were discovered by researchers **[8]**. Using the microbial systems for the production of L-asparaginase has attracted the attention owing to its low cost and large quantity production by submerged and solid-state fermentation [33, 34].

2.5.1. Bacterial sources

L-asparaginase can be produced from both Gram-positive and Gram-negative bacterial species recovered from marine and terrestrial environment **[35]**. Regarding L-asparaginase,

Gram-positive bacteria have attracted less attention when compared to Gram-negative **[16]**. The intensively studied species of both Gram-negative and Gram-positive classes, as L-asparaginase producers, are listed in **table 1**.

2.5.2. Fungal source

Along with the bacteria, there is another powerful source of L-asparaginase. Many side effects associated with bacterial asparaginase, which contradicts their application. This obstacle requires an approach for discovering a new source for L-asparaginase. In contrast to bacteria, fungi are more closely associated with humans. Therefore, fungal L-asparaginase will have less opportunity for immunological reaction **[56]**. In addition, fungal asparaginase has great importance, as it is an extracellular enzyme. Different L-asparaginase-producing fungi are being mentioned as follows: *Alternaria* sp. **[57]**, *Aspergillus nidulans* **[58]**, *Aspergillus niger* **[59]**, *Aspergillus oryzae* **[60]**, *Aspergillus tamarii* **[61]**, *Aspergillus terreus* **[62]**, *Cylidrocapron obtusisporum* **[63]**, *Mucor* sp.**[64]** *and Fusarium roseum* **[65]**.

2.5.3. Actinomycetes source

Actinomycetes also produce L-asparaginase. Actinomycetes are broadly abundant in water and soil, but the ones that presented in living animals particularly in fishes are considered to possess high enzymatic activity. As well as, actinomycetes are found to be a good source of L-asparaginase when compared to fungi and bacteria **[66]**. L-asparaginase-producing actinomycetes as *Actinomyces* sp. **[67]**, *Streptomyces albidoflavus* **[68]**, *Streptomyces aurantiacus* **[68]**, *Streptomyces collinus* **[69]**, *Streptomyces griseus* **[70]**, *Streptomyces gulbargensis* **[71]**, *Streptomyces karnatakensis* **[72]**, *Streptomyces longsporusflavus* **[33]**, *Streptomyces plicatus* **[73]** and *Streptomyces tendae* **[74]**.

Table 1: Bacterial sources of L-asparaginase

Gram positive bacteria	References	Gram negative bacteria	References
Bacillus circulans	**[36, 37]**	*Acinetobacter calcoaceticus*	**[38]**
Bacillus coagulans	**[8]**	*Azotobacter agilis*	**[8]**
Bacillus sp.	**[39]**	*Serratia marcescens*	**[40]**
Bacillus mesentericus	**[8]**	*Citrobacter sp.*	**[41]**
Bacillus polymyxa	**[42]**	*Escherichia coli*	**[43]**
Bacillus subtilis	**[44]**	*Enterobacter aerogenes*	**[45]**
Bacillus licheniformis	**[46, 47]**	*Vibrio succinogenes*	**[8]**
Corynebacterium glutamicum	**[48]**	*Erwinia cartovora*	**[49]**
Mycobacterium bovis	**[8]**	*Erwinia chrysanthemi*	**[50]**
Staphylococcus sp.	**[51]**	*Helicobacter pylori*	**[52]**
Staphylococcus aureus	**[53]**	*Klebsiella pneumoniae*	**[54]**
Streptococcus albus	**[54]**	*Pseudomonas sp.*	**[55]**

2.5.4. Plant source

L-asparaginase is a good tumor inhibitor, so a number of new sources must be discovered in order to meet the need of the medical industry. Researchers have mentioned that green chilies (*Capsicum annum*) and tamarind (*Tamarindus indica*) can produce an amount of L-asparaginase. An enzyme produced from the green chilies was highly purified up to 400-folds by various techniques and it was detected that enzyme appear in two forms, one of them are found to have a tumor inhibiting effect **[9]**.

2.6. L-asparaginase structure

Many researchers to find out L-asparaginase molecular structure have performed many studies. After isolation of L-asparaginase from various sources, it was mentioned that L-asparaginase usually presents as a tetramer and may appear as monomeric, dimeric and hexameric forms. L-asparaginases from bacterial source mostly possess quaternary and tertiary structures **[75]**. *E. coli* and *Erwinia* sp. L-asparaginases are studied in a good way so their structures at the molecular levels and their information is easily abundant **[76, 77]**. Both *E. coli* and *Erwinia* sp. are identical in the three-dimensional structures **[78]**. *Erwinia carotovora* L-asparaginase existed as two tetramers formed from four similar monomers each. I each monomer, three hundred twenty-seven amino acids gather with 14 α-strands, eight β-helices **[79]**, and two domains, a big N-terminal domain and a small C-terminal domain **[76]**. In between the two adjoining monomers, the active site is located. The tetramer formed from four identical subunits. The entire molecule is observed as a dimer of dimers **[80, 81]**.

2.7. Immunological reaction

By increasing the molecular weight of the protein, higher structure complexity and proteins made of many similar subunits with a molecular weight of > 100 KDa the immunogenicity of the protein becomes higher. Clinical trials have demonstrated that the application of the asparaginase molecule, which comprises four similar subunits and has about 140 KDa molecular weight leads to the production of certain antibodies in numerous patients. The antibody reactions range from systemic anaphylaxis to localized erythema and pain at the site of injection and may be followed by a high rate of inactivation to the enzyme. In some reports, the asparaginase is inactivated rapidly in patients who don't show hypersensitivity clinical signs which are called ‘silent inactivation' **[82]**. Killander *et al*. tested L-asparaginase from *E. coli* in two sets including 57 children and 57 adults and revealed that in the adults there were the more frequent appearance of hypersensitivity reactions and a reduction in asparaginase activity in addition to specific antibodies than in the children **[83]**. As asparaginases from various bacterial strains are accessible which demonstrate only restricted cross-reactivity, regimen continuation in case of immunological reactions to one preparation of asparaginase may be conceivable by choosing an enzyme from a various biological source **[12]**. As overall, proteins are immunogenic when the molecular weight higher than 6 kDa **[7]**.

2.8. Industrial production of L-asparaginase and process optimization

Many parameters need to be taken into consideration for the industrial production of L-asparaginase, intending to a higher production and cost-effective process. The pH, temperature, type, and concentration of carbon and nitrogen sources, aeration, fermentation time, and, particularly, the biological agent, have an extraordinary impact on the process **[84]**. As mentioned before, many microorganisms are displayed as L-asparaginase producers; however, bacteria *E. coli* and *Erwinia chrysanthemi* are the

present main microbial sources for industrial production in the pharmaceutical region, while the fungus *Aspergillus oryzae* is the most commonly used in the food industry **[60, 85]**. **Fig. 4** shows a schematic diagram of the industrial procedures for L-asparaginase production.

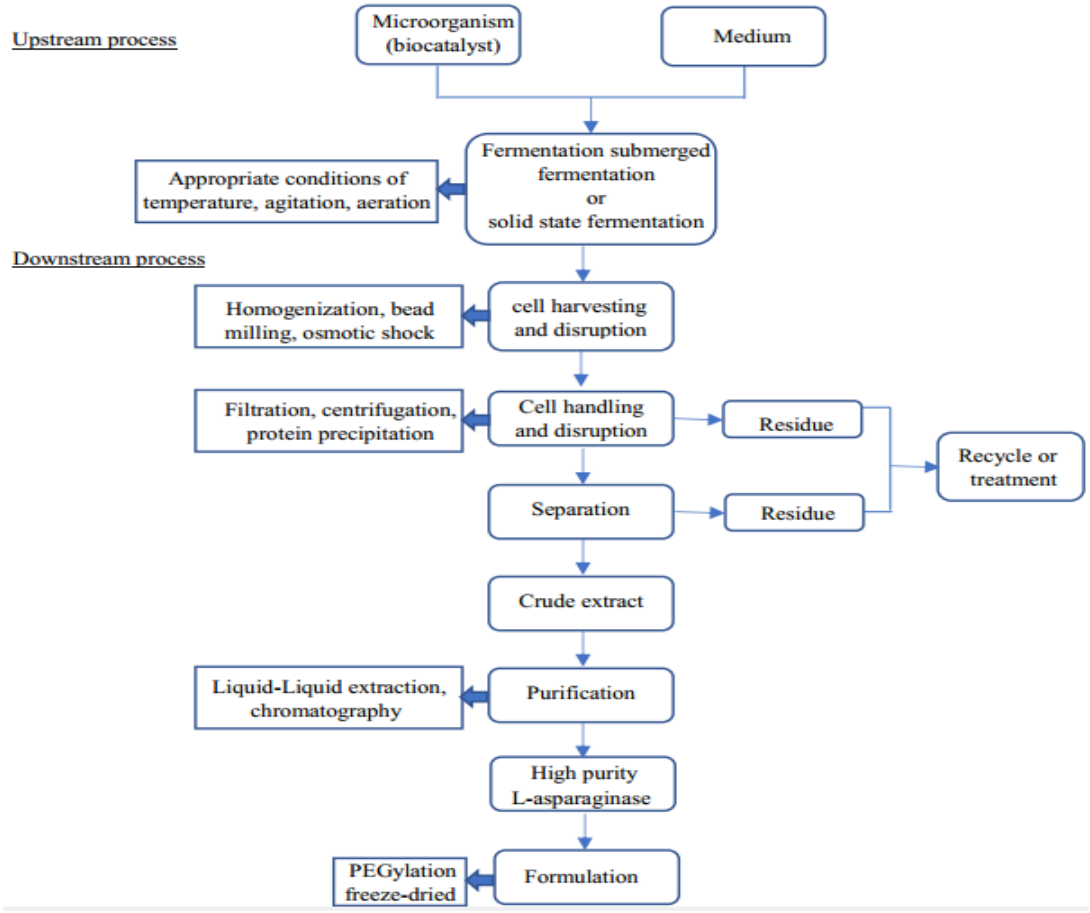

Fig. 4. Schematic representation for an industrial process for L-asparaginase production **[101]**.

For L-asparaginase production, various types of culture media have been investigated. Nonetheless, carbon and nitrogen sources are the most affecting parts of the medium. For example, different investigations have presented that best nitrogen source for reaching high yields are L-asparagine, L-glutamine and L-proline **[86-89]** and glucose is found to be the most common carbon source, as well as other sources such as starch and maltose **[87, 90, 91]**. By studying the different concentration of glucose, it displayed catabolic repression on enzyme production. Glucose at 1% totally represses the enzyme activity while at 0.1 %, it presented marginally stimulatory impact on enzyme production **[4]**. The production of L-asparaginase was observed by utilizing inorganic nitrogen sources such as ammonium sulfate, ammonium chloride, and ammonium nitrate as well as organic sources such as peptone, yeast extract, and beef extract. The optimum productivity has been reported by using 0.5% yeast extract and 0.5% ammonium sulfate. Consequently, yeast extract and ammonium sulfate have been considered as the

best nitrogen sources **[92]**. As aeration and agitation were the influencing parameters for the enzyme production, low aeration and agitation gave the higher yield production while complete aerobic and anaerobic conditions lowered the yield.

Various techniques stated for production and optimization of L-asparaginase from different microorganisms incorporate solid-state fermentation (SSF) and submerged fermentation (SmF) **[93]**. Conditions of the process differ from organism to another for enzyme production, and it can be formed constitutively or after induction **[94]**. Production of L-asparaginase using submerged fermentation was performed by Moorthy *et al*. **[95]**. The culture conditions were optimized for the higher production of the enzyme. Two carbon sources, glucose and maltose, were utilized for the enzyme production. Glucose provided better outcomes. The enzyme was activated by $MgCl_2$ and inhibited by EDTA. Nutritional demands for L-asparaginase production from *Fusarium* species was examined by Tippani and Sivadevuni **[89]**, using submerged fermentation. Glucose was found to be the best carbon source with *Fusarium semitectum* (328 IU/mL), *Fusarium moniliforme* (300 IU/mL) and *Fusarium oxysporum* (210 IU/mL), while lactose was the second best carbon source for *Fusarium oxysporum* (178 IU/mL) and *Fusarium semitectum* (218 IU/mL) and for *Fusarium moniliforme* mannose was the best one (213 IU/mL) **[4]**. Nevertheless, solid state fermentation has some advantages including high yield, cost-effective, low energy input, simple fermentation media, and low water utilization **[96, 97]**.

Solid-state fermentation has been developed as an effective technology for the production of microorganisms' products using raw materials of low cost. An approach was performed by Hosamani *et al*., for optimization of L-asparaginase production by *Fusarium equiseti* utilizing the soya bean under solid state fermentation (SSF), soya bean provided one of the best L-asparaginase yields **[92]**. The production began at 24 h till maximum production at 48 h and then significantly decreased by increasing the incubation time **[92]**. For *Erwinia carotovora*, the optimization was performed using Response Surface Methodology (RSM) [Central Composite Rotatable Design (CCRD)] to test the influence of combination for the three variables. Yeast Extract, Maltose, and L-asparagine, which were, detected earlier using ‘one-factor-at-a time' attempt by them. Within the three variables, yeast extract was found to be significant of maximum productivity 107.46 IU. **[98]**. M. Sunitha *et al*. utilized the Plackett-Burman design to detect the media components controlling the enzyme productivity. They utilized the *Bacillus cereus* MNTG-7 as the source **[99]**. *Streptomyces gulbargensis* was improved by mutagenesis, which enabled isolation of strains that could produce a high yield of the enzyme. The mutant revealed the maximum L-asparaginase productivity (44.7 IU) at 120 h of fermentation via utilizing ground cake extract medium. Abdel-Fattah *et al*. have studied L-asparaginase production by *Pseudomonas aeruginosa* in solid-state culture. Reaction conditions were optimized using Plackett-Burman design **[33]**. The most significant factors were pH, casein hydrolysate and corn-steep liquor and their optimal values as stated by Box-Behnken design were found to be pH 7.9, casein hydrolysate 3.11%, and corn-steep liquor 3.6% **[100]**.

3. Conclusion

This review article highlights miscellaneous applications of L-asparaginase on the commercial level including medical or food industries as well as improvements of its production processes. Several reports have emphasized on the promising role of L-asparaginase in cancer

therapy. Furthermore, L-asparaginase has been introduced in the food industry for the elimination of the carcinogenic compound (acrylamide). In spite of its availability from different sources, microbial L-asparaginase has attracted the attention of the scientists due to its cost-effectiveness and ease of the production. Owing to its commercial value, different approaches have been implemented to optimize L-asparaginase production for higher microbial yields on the industrial level.

Conflict of Interest

The authors declare no conflict of interest.

4. References

1. Nigam PS. Microbial enzymes with special characteristics for biotechnological applications. Biomolecules. 2013; 3(3):597-611.

2. Periasamy Anbu, Subash CB Gopinath, Arzu Coleri Cihan, Bidur Prasad Chaulagain. Microbial enzymes and their applications in industries and medicine. Biomed Res int. 2013; 2013:1-13.

3. Anbu P, S.C.B. Gopinath, A.C. Cihan, B.P. Chaulagain. Strategies to characterize fungal lipases for applications in medicine and dairy industry. Biomed Res int. 2013:1-2.

4. Sinha R, Singh HR, Jha SK. Microbial L-asparaginase: present and future prospective. Int J Innov Res Sci Eng. 2013; 2(11):7031-51.

5. Bansal S, Srivastava A, Mukherjee G, Pandey R, Verma AK, Mishra P, et al. Hyperthermophilic asparaginase mutants with enhanced substrate affinity and antineoplastic activity: structural insights on their mechanism of action. FASEB J. 2012; 26(3):1161-71.

6. Piatkowska-Jakubas B, Krawczyk-Kuliś M, Giebel S, Adamczyk-Cioch M, Czyz A, Lech ME, et al. Use of L-asparaginase in acute lymphoblastic leukemia: recommendations of the Polish Adult Leukemia Group. Pol Arch Med Wewn. 2008;118(11):664-9.

7. Krishnapura PR, Belur PD, Subramanya S. A critical review on properties and applications of microbial l-asparaginases. Crit Revi Microbiol. 2016;42(5):720-37.

8. Batool T, Makky EA, Muna Jalal, Mashitah M Yusoff. A comprehensive review of L-asparaginase and its applications. Appl Biochem Biotechnol. 2016; 178(5):900-23.

9. Kumar J, S, Pasrija D, Sinha RK, Singh HR, Nigam VK, Vidyarthi AS. Microbial L-asparaginase: a review on current scenario and future prospects. Int J Pharm Sci and Res. 2012; 3(9):3076-90.

10. Souza PM, de Freitas MM, Cardoso SL, Pessoa A, Guerra ENS, Magalhães PO. Optimization and purification of l-asparaginase from fungi: A systematic review. Crit Rev Oncol Hematol. 2017;120:194-202.

11. Prager MD, Bachynsky N. Asparagine synthetase in asparaginase resistant and susceptible mouse lymphomas. Biochem Biophys Res Comm. 1968; 31(1):43-7.

12. Boos J. Use of l-asparaginase in childhood ALL. Crit Rev Oncol Hematol. 1998; 2(28):97-113.

13. Ohnuma T, Bergel F, Bray R. Enzymes in cancer: asparaginase from chicken liver. Biochem J. 1967; 103(1):238.

14. Richards NG, Kilberg MS. Asparagine synthetase chemotherapy. Annu Rev Biochem. 2006; 75:629-54.

15. Ueno T, Ohtawa K, Mitsui K, Kodera Y, Hiroto M, Matsushima A. Cell cycle arrest and apoptosis of leukemia cells induced by L-asparaginase. Leukemia. 1997; 11(11):1858.

16. Asthana N, Azmi W. Microbial L-asparaginase: A potent antitumor enzyme. ind J Biotechnol. 2003;2:184-94.

17. Mottram DS, Wedzicha BL, Dodson AT. Food chemistry: acrylamide is formed in the Maillard reaction. Nature. 2002; 419(6906):448.

18. Pedreschi F, Mariotti MS, Granby K. Current issues in dietary acrylamide: formation, mitigation, and risk assessment. J Sci Food Agric. 2014;94(1):9-20.

19. Yu LL, Wang S, Sun B-G. Food safety chemistry: toxicant occurrence, analysis, and mitigation: CRC press; 2014.

20. Xu F, Oruna-Concha M-J, Elmore JS. The use of asparaginase to reduce acrylamide levels in cooked food. Food Chem. 2016; 210:163-71.

21. Sadd PA, Hamlet CG, Liang L. Effectiveness of methods for reducing acrylamide in bakery products. J agric food chem. 2008; 56(15):6154-61.

22. Bartkiene E, Jakobsone I, Juodeikiene G, Vidmantiene D, Pugajeva I, Bartkevics V. Study on the reduction of acrylamide in mixed rye bread by fermentation with bacteriocin-like inhibitory substances producing lactic acid bacteria in combination with *Aspergillus niger* glucoamylase. Food Control. 2013; 30(1):35-40.

23. Bartkiene E, Jakobsone I, Juodeikiene G, Vidmantiene D, Pugajeva I, Bartkevics V. Effect of lactic acid fermentation of lupine wholemeal on acrylamide content and quality characteristics of wheat-lupine bread. Int j food sci nutr. 2013; 64(7):890-6.

24. Kamkar A, Qajarbeygi P, Jannat B, Haj Hosseini Babaei A, Misaghi A, Molaee Aghaee E. The inhibitory role of autolyzed yeast of *Saccharomyces cerevisiae*, vitamins B3 and B6 on acrylamide formation in potato chips. Toxin Rev. 2015; 34(1):1-5.

25. Boegl p, Zuzana C, Eugen K. Impact of L-asparaginase on acrylamide content in potato products. J Food Nutr Res. 2006;45(4):141-6.

26. Parker JK, Balagiannis DP, Higley J, Smith G, Wedzicha BL, Mottram DS. Kinetic model for the formation of acrylamide during the finish-frying of commercial French fries. J Agr Food Chem. 2012;60(36):9321-31.

27. Zyzak DV, Sanders RA, Stojanovic M, Tallmadge DH, Eberhart BL, Ewald DK, et al. Acrylamide formation mechanism in heated foods. J Agr Food Chem. 2003; 51(16):4782-7.

28. Vass M, Amrein T, Schonbachler B, Escher F, Amado R. Ways to reduce the acrylamide formation in cracker products. Czech J Food Sci. 2004;22(I):19.

29. Verma N, Kumar K, Kaur G, Anand S. *E. coli* K-12 asparaginase-based asparagine biosensor for leukemia. Artif Cells Blood Substit Biotechnol. 2007; 35(4):449-56.

30. Verma N, Bansal M, Kumar S. Whole cell based miniaturized fiber optic biosensor to monitor L-asparagine. Adv Appl Sci Res. 2012; 3(2):809-14.

31. Borek D, Jaskólski M. Sequence analysis of enzymes with asparaginase activity. Acta Biochim Pol. 2001; 48:893-902.

32. Bruneau L, Chapman R, Marsolais F. Co-occurrence of both L-asparaginase subtypes in Arabidopsis: At3g16150 encodes a K+-dependent L-asparaginase. Planta. 2006; 224(3):668-79.

33. Abdel-Fattah YR, Olama ZA. L-asparaginase production by *Pseudomonas aeruginosa* in solid-state culture: evaluation and optimization of culture conditions using factorial designs. Process Biochem. 2002; 38(1):115-22.

34. Kumar S, Dasu VV, Pakshirajan K. Purification and characterization of glutaminase-free L-asparaginase from *Pectobacterium carotovorum* MTCC 1428. Bioresour Technol. 2011; 102(2):2077-82.

35. Izadpanah QF, Javadpour S, Malekzadeh K, Tamadoni Jahromi S, Rahimzadeh M. Persian gulf is a bioresource of potent L-asparaginase producing bacteria: Isolation & molecular differentiating. Int J Environ Res. 2014; 8(3):813-8.

36. Hymavathi M, Sathish T, Rao CS, Prakasham R. Enhancement of L-asparaginase production by isolated *Bacillus circulans* (MTCC 8574) using response surface methodology. Appl Biochem Biotechnol. 2009; 159(1):191-8.

37. Prakasham R, Hymavathi M, Rao CS, Arepalli S, Rao JV, Kennady PK, et al. Evaluation of antineoplastic activity of extracellular asparaginase produced by isolated *Bacillus circulans*. Appl Biochem Biotechnol. 2010; 160(1):72.

38. Joner PE. Purification and properties of l-asparaginase B from *Acinetobacter calcoaceticus*. Biochimica et Biophysica Acta (BBA)-Enzymology. 1976;438(1):287-95.

39. Mohapatra B, Sani R, Banerjee U. Characterization of L-asparaginase from *Bacillus* sp. isolated from an intertidal marine alga (*Sargassum* sp.). Lett Appl Microbiol. 1995; 21(6):380 3.

40. Rowly B, Wriston J. L-asparaginase from *Serratia marcescens*. Biochem Biophys Res Commun. 1967; 28:160-71.

41. Shoshana B, Banks G, Skarstedt M, Fleming A, Bettelheim K, Connors T. The properties and large-scale production of L-asparaginase from *Citrobacter*. Microbiology. 1975; 91(1):1-16.

42. Nefelova M, Ignatov S, Chigalenchik A, Vinogradov B, Egorov N. Biosynthesis of L-asparaginase-2 by cultures of *Bacillus polymyxa* var. Ross. Prikladnaia biokhimiia i mikrobiologiia. 1978; 14(4):510-4.

43. Netrval J. Stimulation of L-asparaginase production in *Escherichia coli* by organic and amino acids. Folia microbiol. 1977;22(2):106-16.

44. Fisher SH, Wray LV. *Bacillus subtilis* 168 contains two differentially regulated genes encoding L-asparaginase. J Bacteriol. 2002; 184(8):2148-54.

45. Mukherjee J, Majumdar S, Scheper T. Studies on nutritional and oxygen requirements for production of L-asparaginase by *Enterobacter aerogenes*. Appl Microbiol Biotechnol. 2000; 53(2):180-4.

46. Golden KJ, Bernlohr RW. Nitrogen catabolite repression of the L-asparaginase of *Bacillus licheniformis*. J Bacteriol. 1985; 164(2):938-40.

47. Sudhir AP, Dave BR, Prajapati AS, Panchal K, Patel D, Subramanian R. Characterization of a recombinant glutaminase-free l-asparaginase (ansa3) enzyme with high catalytic activity from *Bacillus licheniformis*. Appl Biochem Biotechnol. 2014; 174(7):2504-15.

48. Mesas JM, Gil JA, Martín JF. Characterization and partial purification of L-asparaginase from *Corynebacterium glutamicum*. Microbiology. 1990; 136(3):515-9.

49. Maladkar N, Singh V, Naik S. Fermentative production and isolation of L-asparaginase from *Erwinia carotovora*, EC-113. Hindustan Antibiot bull. 1993; 35(1-2):77-86.

50. Moola ZB, Scawen M, Atkinson T, Nicholls D. *Erwinia chrysanthemi* L-asparaginase: epitope mapping and production of antigenically modified enzymes. Biochem J. 1994; 302(3):921-7.

51. Mikucki J, Szarapińska-Kwaszewska J, Krzemiński Z. Factors influencing L-asparaginase production by *Staphylococci*. Zentralbl Bakteriol Parasitenkd Infektionskr Hyg. 1977; 132(2):135-42.

52. Stark R, Suleiman M-S, Hassan I, Greenman J, Millar M. Amino acid utilisation and deamination of glutamine and asparagine by *Helicobacter pylori*. J Med Microbiol. 1997;46(9):793-800.

53. Rozalska M, Mikucki J. *Staphylococcal* L-asparaginase: catabolic repression of synthesis. Acta Microbiologica Polonica. 1992; 41(3-4):145-50.

54. Reddy K. Effect of C and N sources on asparaginase production by bacteria. Ind J Microbiol 1990;30(1):81-3.

55. Zhang N, Clarke F, Di Trapani G, Keough D, Beacham I. Guinea pig serum L-asparaginase: purification, and immunological relationship to liver L-asparaginase and serum L-asparaginases in other mammals. Comp Biochem Physiol B, Biochem Mol Biol. 1995; 112(4):607-12.

56. Shrivastava A, Khan AA, Shrivastav A, Jain SK, Singhal PK. Kinetic studies of L-asparaginase from *Penicillium digitatum*. Prep Biochem Biotechnol. 2012; 42(6):574-81.

57. Gummadi C. Screening and isolation of novel glutaminase free L-asparaginase from fungal endophytes. Res J Microbiol. 2014; 9(4):163-76.

58. Saxena RK, Sinha U. L-asparaginase and glutaminase activities in the culture filtrates of *Aspergillus nidulans*. Curr sci. 1981; 50:218-9.

59. Mishra A. Production of L-asparaginase, an anticancer agent, from *Aspergillus niger* using agricultural waste in solid state fermentation. Appl Biochem Biotechnol. 2006; 135(1):33-42.

60. Hendriksen HV, Kornbrust BA, Østergaard PR, Stringer MA. Evaluating the potential for enzymatic acrylamide mitigation in a range of food products using an asparaginase from *Aspergillus oryzae*. J Agr Food Chem. 2009; 57(10):4168-76.

61. Sarquis MIdM, Oliveira EMM, Santos AS, Costa GLd. Production of L-asparaginase by filamentous fungi. Mem Inst Oswaldo Cruz. 2004; 99(5):489-92.

62. Gurunathan B, Sahadevan R. Production of L-asparaginase from natural substrates by *Aspergillus terreus* MTCC 1782: Effect of substrate, supplementary nitrogen source and L-asparagine. Int J Chem React Eng. 2009;7(1):doi.org/10.2202/1542-6580.2050.

63. Raha S, Roy S, Dey S, Chakrabarty S. Purification and properties of an L-asparaginase from *Cylindrocarpon obtusisporum* MB-10. Biochem Int. 1990; 21(6):987-1000.

64. Mohapatra B, Bapuji M, Banerjee U. Production and properties of L-asparaginase from Mucor species associated with a marine sponge (*Spirastrella* sp.). Cytobios. 1997; 92(370-371):165-73.

65. Nakahama K, Imada A, Igarasi S, Tubaki K. Formation of L-asparaginase by *Fusarium* species. Microbiology. 1973;75(2):269-73.

66. Sahu MK, Sivakumar K, Poorani E, Thangaradjou T, Kannan L. Studies on L-asparaginase enzyme of *actinomycetes* isolated from estuarine fishes. J Environ Biol. 2007; 28(2):465.

67. Narayana K, Kumar K, Vijayalakshmi M. L-asparaginase production by *Streptomyces albidoflavus*. Ind J Microbiol. 2008; 48(3):331-6.

68.Gupta N, Mishra S, Basak U. Occurrence of *Streptomyces aurantiacus* in mangroves of Bhitarkanika. Malays J Microbiol. 2007;3(2):7-14.

69. Mostafa S, Salama M. L-asparaginase-producing *Streptomyces* from the soil of Kuwait. Zentralbl Bakteriol Naturwiss. 1979; 134(4):325-34.

70. Dejong PJ. L-Asparaginase production by *Streptomyces griseus*. Appl Microbiol. 1972; 23(6):1163-4.

71. Amena S, Vishalakshi N, Prabhakar M, Dayanand A, Lingappa K. Production, purification and characterization of L-asparaginase from *Streptomyces gulbargensis*. Braz J Microbiol. 2010; 41(1):173-8.

72. Mostafa S. Activity of L-asparaginase in cells of *Streptomyces karnatakensis*. Zentralbl Bakteriol Naturwiss. 1979; 134(4):343-51.

73. Koshy A, Dhevendaran K, Georgekutty M, Natarajan P. L-asparaginase in *Streptomyces plicatus* isolated from the alimentary canal of the fish, Gerres filamentosus (Cuvier). J Mar Biotechnol. 1997; 5:181-5.

74. Kavitha A, Vijayalakshmi M. Optimization and Purifi cation of L-Asparaginase Produced by *Streptomyces tendae* TK-VL_333. Z Naturforsch C. 2010; 65(7-8):528-31.

75. Ramya L, Doble M, Rekha V, Pulicherla K. L-Asparaginase as potent anti-leukemic agent and its significance of having reduced glutaminase side activity for better treatment of acute lymphoblastic leukemia. Appl Biochem Biotechnol. 2012;167(8):2144-59.

76. Aghaiypour K, Wlodawer A, Lubkowski J. Structural basis for the activity and substrate specificity of *Erwinia chrysanthemi* L-asparaginase. Biochemistry. 2001; 40(19):5655-64.

77. Swain AL, Jaskólski M, Housset D, Rao J, Wlodawer A. Crystal structure of *Escherichia coli* L-asparaginase, an enzyme used in cancer therapy. Proc Nat Acad Sci. 1993; 90(4):1474-8.

78. Lubkowski J, Dauter M, Aghaiypour K, Wlodawer A, Dauter Z. Atomic resolution structure of *Erwinia chrysanthemi* L-asparaginase. Acta Cryst D. 2003; 59(1):84-92.

79. Kozak M, Jaskólski M, Rohm K. Preliminary crystallographic studies of Y25F mutant of periplasmic *Escherichia coli* L-asparaginase. Acta Biochim Pol. 2000;47(3):807-14.

80. Jaskolski M, Kozak M, Lubkowski J, Palm G, Wlodawer A. Structures of two highly homologous bacterial l-asparaginases: a case of enantiomorphic space groups. Acta Cryst D. 2001; 57(3):369-77.

81. Sanches M, Barbosa JaAR, De Oliveira RT, Abrahão Neto J, Polikarpov I. Structural comparison of *Escherichia coli* L-asparaginase in two monoclinic space groups. Acta Cryst D. 2003; 59(3):416-22.

82. Ahlke E, Nowak-Go¨ Ttl U, Schulze-Westhoff P, Werber G, Bo¨ Rste H, WU¨ Rthwein G, et al. Dose reduction of asparaginase under pharmacokinetic and pharmacodynamic control during induction therapy in children with acute lymphoblastic leukemia. Brit J Haematol. 1997; 96(4):675-81.

83. Killander D, Dohlwitz A, Engstedt L, Franzen S, Gahrton G, Gullbring B, et al. Hypersensitive reactions and antibody formation during L-asparaginase treatment of children and adults with acute leukemia. Cancer. 1976; 37(1):220-8.

84. Gurunathan B, Sahadevan R. Optimization of culture conditions and bench-scale production of L-asparaginase by submerged fermentation of *Aspergillus terreus* MTCC 1782. J Microbiol Biotechnol. 2012;22(7):923-9.

85. Lopes AM, Oliveira-Nascimento Ld, Ribeiro A, Tairum Jr CA, Breyer CA, Oliveira MAd, et al. Therapeutic l-asparaginase: upstream, downstream and beyond. Crit Rev Biotechnol. 2017; 37(1):82-99.

86. Baskar G, Renganathan S. Evaluation and screening of nitrogen source for l-asparaginase production by *Aspergillus terreus* MTCC 1782 using latin square design. Res J Math Stat. 2009; 1(2):55-8.

87. Aida M, Farag, Sahar W, Hassan, Ehab A, Beltagy, Mohamed A, El-Shenawy. Optimization of production of anti-tumor l-asparaginase by free and immobilized marine *Aspergillus terreus*. Egypt J Aquat Res. 2015; 41(4):295-302.

88. Kiranmayi MU, Poda S, Vijayalakshmi M. Production and optimization of L-asparaginase by an *actinobacterium* isolated from Nizampatnam mangrove ecosystem. J Environ Biol. 2014;35(5):799.

89. Tippani R, Sivadevuni G. Nutritional factors effecting the production of L-asparaginase by the *Fusarium* sp. Afr J Biotechnol. 2012; 11(15):3692-6.

90. Vijayalakshmi M, Kavitha K, Akilandeswari K. Production of bioactive enzyme L-asparaginase from fungal isolates of water sample through submerged fermentation. Int JPharm Pharm Sci. 2012; 4(4):363-6.

91. Varalakshmi V, Raju JK. Optimization of l-asparaginase production by *Aspergillus terreus* mtcc 1782 using bajra seed flour under solid state fermentation. Int J Res Eng Technol. 2013; 2(09):121-9.

92. Hosamani R, Kaliwal B. L-asparaginase an anti-tumor agent production by *Fusarium equiseti* using solid state fermentation. Int J Drug Discov. 2011;3(2):88-99.

93. Nagarethinam S, Naik AN, Udupa N, Rao VJ, Vanathi MB. Microbial L-Asparaginase and its future prospects. Asian J MedRes. 2012; 1(4):159-68.

94. Ahmad N, Pandit NP, Maheshwari SK. L-asparaginase gene-a therapeutic approach towards drugs for cancer cell. Int J Biosci. 2012; 2(4):1-11.

95. Moorthy V, Ramalingam A, Sumantha A, Shankaranaya RT. Production, purification, and characterization of extracellular L-asparaginase from a soil isolate of *Bacillus* sp. Afr J Microbiol Res. 2010; 4(18):1862-7.

96. Mishra A, Das MD. Effect of pH on simultaneous saccharification and isomerization by glucoamylase and glucose isomerase. Appl Biochem Biotechnol. 2002; 102(1-6):193-9.

97. Mukherjee P, Pandey A, Selvakumar P, Ashakumary L, Gurusamy P. X-ray diffraction studies on solid state fermentation for the production of glucoamylase using *Aspergillus niger* NCIM 1248. J Sci Ind Res. 1998; 57(10-11):583-6.

98. Deokar VD, Vetal MD, Lambert Rodrigues. Production of intracellular L-asparaginase from *Erwinia carotovora* and its statistical optimization using response surface methodology (RSM). Int J Chem Sci. 2010; 1:25-36.

99. Sunitha M, Ellaiah P, Devi RB. Screening and optimization of nutrients for L-asparaginase production by *Bacillus cereus* MNTG-7 in SmF by plackett-burmann design. Afr JMicrobiol Res. 2010; 4(4):297-303.

100. El-Bessoumy AA, Sarhan M, Mansour J. Production, isolation, and purification of L-asparaginase from *Pseudomonas aeruginosa* 50071 using solid-state fermentation. BMB Reports. 2004; 37(4):387-93.

101. Cachumba JJM, Antunes FAF, Peres GFD, Brumano LP, Dos Santos JC, Da Silva SS. Current applications and different approaches for microbial l-asparaginase production. Braz J Microbiol. 2016;47:77-85.

102. Uygun M, Jurado-Sánchez B, Uygun DA, Singh VV, Zhang L, Wang J. Ultrasound-propelled nanowire motors enhance asparaginase enzymatic activity against cancer cells. Nanoscale. 2017; 9(46):18423-9.