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SCREENING OF CULTURE MEDIA FOR LYSINE PRODUCTION BY *CORYNEBACTERIUM GLUTAMICUM* IIB646

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ABSTRACT

Lysine amino acid is essential for humans and mammals can be produced in fermentation processes by using different microorganisms. Fermentation process for synthesis of L-lysine contributes about 80% of total annually produced worldwide. In this research work L-lysine was produced by using local bacterial isolate i.e. *Corynebacterium glutamicum* IIB646 in submerged fermentation process. Here fifteen different culture/fermentation media were utilized and optimized for the synthesis of lysine amino acid. Lysine was synthesized in different concentration in these different fifteen culture media. Maximum L-lysine production amount was observed in fermentation media number thirteen (M13). M13 synthesized maximum 5.5 g/L lysine amount. This M13 medium consists of ammonium sulphate as nitrogen source, glucose as carbon source, calcium carbonate, manganese chloride, casamino acids and biotin as nutritional components. Culture medium M13 was then further supplemented with various sources of carbon and nitrogen in different quantities to enhance the synthesis of Lysine amount. Finally glucose as a best carbon source and amm. sulphate as suitable nitrogen source was screened. Hence maximum 7.5 g/L lysine was synthesized after fermentation period of 72 hours in the presence of M13 culture medium, which was supplemented with optimized concentrations of 7% glucose as carbon source and 2.5% of ammonium sulphate as a source of nitrogen.

Key-words: L-lysine, Culture media, Submerged Fermentation, Optimization.

INTRODUCTION

Amino acids are important for both the human nutrition and animal feed (Bercovici and Fuller, 2007). In the second half of twentieth century its production technology made enormous progress due to increase in its market demand. Enzymatic and fermentation technologies are two main biotechnological processes which are used for production and extension of the amino acid industries. Lysine, threonine, methionine and tryptophan have the highest share about 56 % of total amino acids in the market, its about 4.6 US$ billion (Leuchtenberger *et al*., 2005). Today, amino acids synthesized by fermentation techniques are considered the important biotechnological methods (Ikeda, 2003). L-lysine amino acid is one of the commercially important and very essential to synthesize in large scale in the industry of amino acids. Lysine amino acid can be synthesized in highly pure form i.e. higher than 98 % (Fechter *et al*., 1997).

Optimization of culture medium and conditions can lower the production costs of Lysine at industrial scale. About 80 % of total world market L-lysine is produced by microbial fermentation methods and about 20 % of L-lysine by chemical synthesis techniques. Natural and biological raw materials are used in fermentation process. Organic and inorganic non toxic by-products are synthesized during L-lysine fermentation processes (Anastassiadis, 2007). Different microorganisms including *Brevibacterium flavum* (Ikeda, 2003), *B. lactofermentum* (Fechter *et al*., 1997) and *Corynebacterium glutamicum*, (Anastassiadis, 2007) had been utilized for about 60 years for high scale synthesis of the Lysine amino acid, but *C. glutamicum* was considered as main production organism for the synthesis of Lysine. Lysine can be synthesized during submerged fermentation process by a rod shaped, non sporulating, fast growing and Gram positive *C. glutamicum* (Udaka, 1960; Kinoshita *et al*., 1957). Bacterial strain *C. glutamicum* have ability to synthesize different amino acids including histidine (Araki *et al*., 1974), threonine, methionine (Kase and Nakayama, 1975), valine by Ruklisha *et al*. (2007), serine (Eggeling, 2007), phenylalanine and tyrosine by Ikeda and Katsumata (1992), L-tryptophan (Ikeda, 2006), leucine and isoleucine (Guillout *et al*., 2002). Due to the production of large number of amino acid by *C. glutamicum*, it was considered an important microorganism in the industry of biotechnology. In biotechnology industry production rates of Lysine amount have

been further enhanced by culture optimization processes and microbial strains improvement techniques by using different mutagens (Pfefferle *et al.,* 2003). Submerged fermentation media, temperature conditions, concentrations of glucose as carbon source and nitrogen sources are the factors which plays important role in fermentation processes for synthesis of products. Present study was done to synthesize L-lysine amino acid by using different culture media, carbon, nitrogen sources and by optimizing different concentrations of nutritional components.

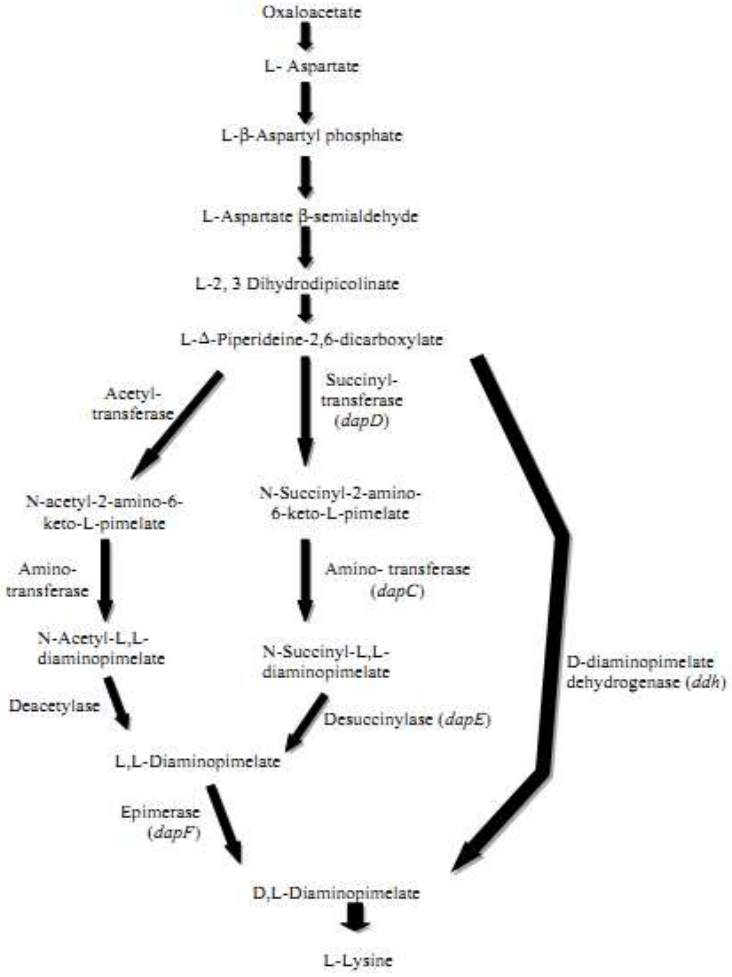

Fig.1. L-lysine Production Pathway.

For optimizing culture media, its different chemical and physical parameters are studied (Adnan *et al.,* 2011; Sassi *et al*., 1996). Lysine producing microorganisms can use different carbon sources, minerals, nitrogen sources and salts in the fermentation processes. Each microbial strain needs suitable and optimum cultural conditions to enhance product yield of amino acids. So it is essential to examine the effects of culture conditions including temperature, pH, agitation and incubation period on the product yield (Eggeling, 1994).

Present study aims to screen and optimizing different fermentation/cultural media for increasing L-Lysine synthesis by *Corynebacterium glutamicum* strain IIB646 through the submerged culturing in shaking flasks so that the production process can be made economical by selecting a cost effective fermentation medium.

MATERIAL AND METHODS

Fermentation experiment

Fresh inoculum of *Corynebacterium glutamicum* IIB646 was used in submerged fermentation experiments in small conical flasks containing 25 mL nutrient broth medium. The Conical flask was then kept at 30ºC temperature in shaking incubator for 24-48hrs. After fermentation experiments synthesized L-lysine concentration was measured from the fermentation broth.

Fermentation Media

M1. Glucose 60 g/L, amm. sulphate 40 g/L, urea 20 g/L, potassium phosphate 0.6 g/L, ferrous sulphate 0.02 g/L, $CaCl_2$ 0.04 g/L, copper sulphate 0.3 g/L, manganese sulphate monohydrate 0.02 g/L, zinc sulphate heptahydrate 1.0 mg/L, nickel chloride 0.03 mg/L and Biotin 250 µg/L (Modified by Broer *et al*., 1993).

M2. Clarified cane molasses 20g/L, $CaCO_3$ 25 g/L, NaCl 2.5 g/L and potassium hydrogen phosphate 0.4g/L (Nelofer *et al*., 2007).

M3. Glucose 10.5 g/L, yeast extract 2.7 g/L, peptone 08 g/L, meat extract 4.3 g/L, NaCl 2.5 g/L (Modified Baulin media).

M4. Sugar 75 g/L, amm. sulphate 5.5 g/L, peptone 10g/L, potassium hydrogen phosphate 0.7 g/L, sodium chloride 3.5 g/L, yeast Extract 5.0g/L, and manganese sulphate 0.6 g/L, (Anastassidis, 2007).

M5. Sucrose 60 g/L, amm. sulphate 40 g/L, magnesium sulphate 0.6 g/L, Urea 2.5 g/L, dipotassium hydrogen phosphate 0.06 g/L, ferrous sulphate 0.02 g/L, NaCl 1.5 g/L and manganese sulphate 15 mg/L (Bathe *et al*., 2004).

M6. Sugar 6.5 g/L, yeast Extract 3.5 g/L, tryptone 6.5 g/L, sodium chloride 6.5 g/L (LB medium Modified).

M7. Glucose 40g/L, amm. sulphate 30 g/L, magnesium sulphate 1.8 g/L, Biotin 110 µg/L, and soya hydrolysate 25 g/L (Oh *et al*., 1993).

M8. Glucose 80 g/L, amm. sulphate 60 g/L, magnesium sulphate heptahydrate 0.6 g/L, manganese sulphate dihydrate 12 mg/L, ferrous sulphate 1.5 mg/L, calcium pentothenate 3 mg/L and cornsteep 50 g/L (Gulbler *et. al*., 1994).

M9. Sugar 40 g/L, yeast extract 2.5g/L, peptone 6.5 g/L, biotin 3 mg/L, ammonium sulphate 40 g/L, potassium di-hydrogen phosphate 0.5g/L, calcium chloride 0.6 g/L, di-potassium phosphate 0.6 g/L (Locally designed).

M10. Sugar 50 g/L, magnesium sulphate 0.5 g/L, urea 5 g/L, potassium dihydrogen phosphate 1.5 g/L, biotin 2 µg/L and thiamine HCl 70 µg/L (Calik *et al*., 2001).

M11. (g/L) Sugar 40, malt extract 3.5, yeast extract 3, peptone 3.5, potassium phosphate 1.5 and magnesium sulphate 1.5 (Rattray and Fox, 1997).

M12. (g/L) Glucose 150, amm. sulphate 35, $CaCO_3$ 30, potassium di-hydrogen phosphate 2.5, ferrous sulphate 0.6, biotin 50 µg/L, magnesium sulphate seven hydrate 0.5, casamino acids 1.2, manganese chloride 0.5 and thiamine 130 µg/L (Shah *et al*., 2012).

M13. Sugar 60 g/L, di-potassium phosphate 0.7 g/L, ammonium sulphate 30g/L, potassium di-hydrogen phosphate 0.7 g/L, magnesium sulphate 6 mg/L, ferrous sulphate 1.5 mg/L, calcium carbonate 10.5 g/L, biotin 20 µg/L, casamino acids 1.5 g/L and thiamine 50 µg/L (Modified; Shah *et al*., 2012).

M14. Corn steep 90 g/L, amm. sulphate 35 g/L, $CaCO_3$ 20 g/L, potassium di-hydrogen phosphate 1.5 g/L, magnesium sulphate 2.2 g/L, ferrous sulphate 0.03 g/L, manganese chloride 2 mg/L, sodium chloride 2.2 g/L, thiamine 200 µg/L and biotin 100 µg/L (Rehman *et al*., 2012)

M15. (g/L). Sugar 95, amm. sulphate 25, magnesium sulphate 0.2, thiamine HCl 200 µg/L, biotin 200 µg/L, potassium phosphate 0.6 (Coello *et al*., 2002).

Analysis of Lysine

Lysine analysis was accomplished by Ninhydrin ferric reagents methods (Hsieh *et al*., 1995). Here 650µL of A reagent (ferric chloride, methylecellosolve, KCl) while 355µl of B reagent (Ninhydrin solution in KCl) added in 20µL of sample. This mixture heated at 100°C for 25 min. Then its temperature was lowered at 25°C and added 100µL of DMSO. Added 5mL deionized water then absorbance was taken at 480nm in a spectrophotometer.

Glucose Analysis

Residual sugar was measured by using DNS method (Miller, 1959).

RESULTS AND DISCUSSION

Fifteen distinct culture media including M1 to M15 were screened in lysine production by *C. glutamicum* IIB646. Capacity of these fifteen fermentation media for L-lysine production was carried in 250 ml of Conical flasks. The process of fermentation experiments was carried out in conical flask at 30˚C for 72 hrs and at shaking speed of 200 rpm. Fig. 2 showed that the IIB646 strain synthesized highest 5.5 g/L Lysine in M13 medium. Moreover this *C. glutamicum* IIB646 produced 4.9 g/L Lysine in M10, 4.5 in M12, 4.1 in M9, 3.4 in M1 and 3.1 g/L of Lysine in M3 and in M8 medium. In other fermentation and culture media, this bacterial strain synthesized lower than 3 g/L Lysine, while 2.3 g/L Lysine synthesized in M7 medium. M13 medium showed greater quantity of

L-lysine yield as a comparison to the other culture media used in this study. This M13 medium was selected for further optimization studies (Fig. 2).

Culture medium M13 had necessary constituents which were suitable for bacterial isolates for L-lysine production. M13 medium consists of Glucose, amm. sulphate, potassium phosphate, calcium carbonate, magnesium sulphate, manganese chloride, ferrous sulphate, casamino acid, thiamine HCl and biotin. These results are in agreements to the study of Oh *et al*. (1993) and Shah *et al*. (2012) who used components of M13 culture medium for increasing L-lysine yield (Fig. 2).

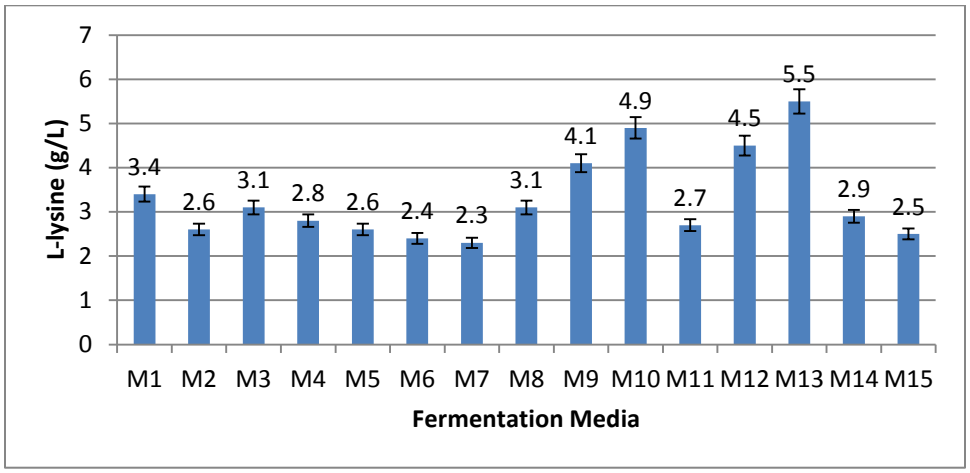

Fig. 2. Lysine production in fermentation media by *Corynebacterium glutamicum* IIB646.

L-lysine synthesis rate was observed for upto 144 hours. Figure 3 represents lysine amount, sugar and cell mass production during fermentation process. Results showed that *C. glutamicum* IIB646 synthesized 1.2 (g/L) of Lysine during 26 hours of incubation period, 3.4 g/L of lysine in 48 hrs, 5.5 g/L in 72 hours and maximum 6.2 (g/L) Lysine during 96 hours of fermentation period. Maximum dry cell mass of 5.2 g/L was obtained in 144 hours of fermentation period (Fig. 3).

After optimum fermentation time period there may be decline in product yield (Cocaign *et al*., 1993). Fermentative yield of L-lysine from bacterial species were being enhanced in about 70 hours to 102 hours (Anastassiadis, 2007). Adnan *et al*., (2011) reported higher lysine yield during 48 hours to 72 hours of fermentation period. Similarly higher lysine yield was reported by Ishii *et al*. (1997) after 72-96 hours of fermentation time period.

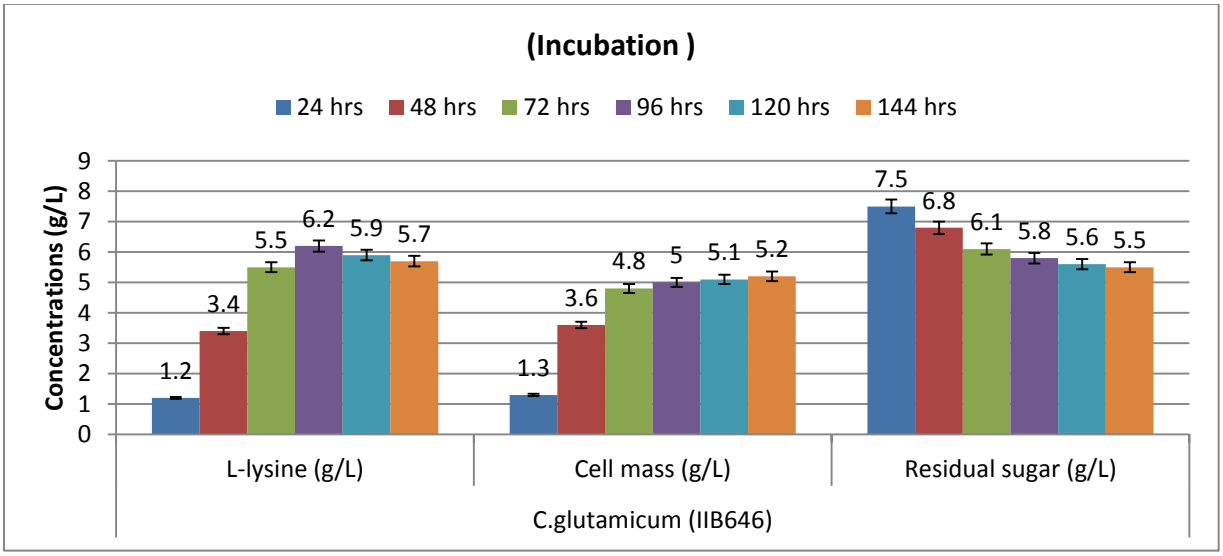

Fig. 3. Lysine production at different incubation time.

Fermentation Conditions:- "Medium: M13; Temperature: 30°C;

Six different carbon sources (sugar, molasses, sucrose, fructose, starch and lactose) were used for Lysine synthesis. Highest 6.4 g/L Lysine synthesized in the fermentation media which contain sugar (glucose) in the form of carbon source. 4.8 g lysine in sucrose, 3.5 g in the Fructose, 5.1 (g/L) of Lysine in the presence of molasses as carbon source, 2.3 g in lactose and 3.2 g/L Lysine was synthesized by a culturing medium which contained starch as a carbon source. Maximum 6.2 g of dry cell masses analyzed in presence of glucose. Sugar utilization during the fermentation experiments was also studied and minimum residual sugar was found in the presence of glucose as a carbon source (Fig. 4).

Carbon source causes major influence on microbial products synthesis. Our results correlate with the study of Nasri *et al.*, (1989), they reported highest Lysine amount in the presence of glucose (as carbon source). Kiefer *et al.*, (2002) and Cerning *et al*. (1994) also reported glucose as a best carbon source for product yield.

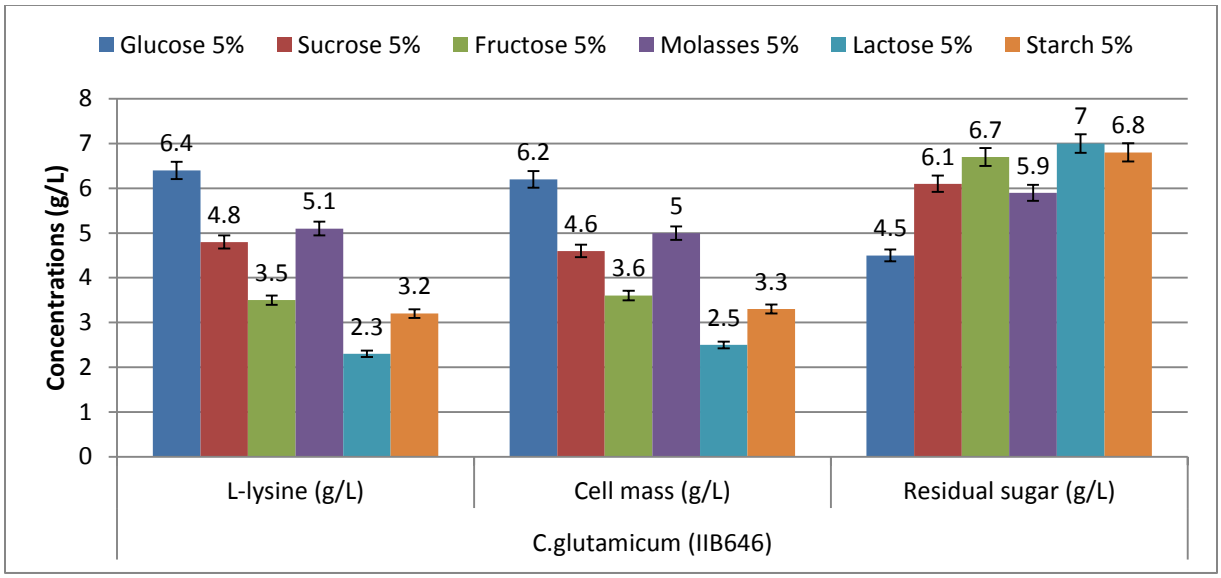

Fig. 4. Lysine production in different carbon sources.

Further 4 to 9% glucose concentrations were optimized and highest 7.1 g/L of Lysine synthesized in 7% glucose. Minimum amount 5.5 g of Lysine produced when medium was supplemented with 4% of glucose. Maximum 6.9 g/L dry cell mass was found with use of 8% glucose and lowest 5.4 g of dry cell mass in existence of 4% of glucose in the fermentation medium was observed (as in Fig. 5).

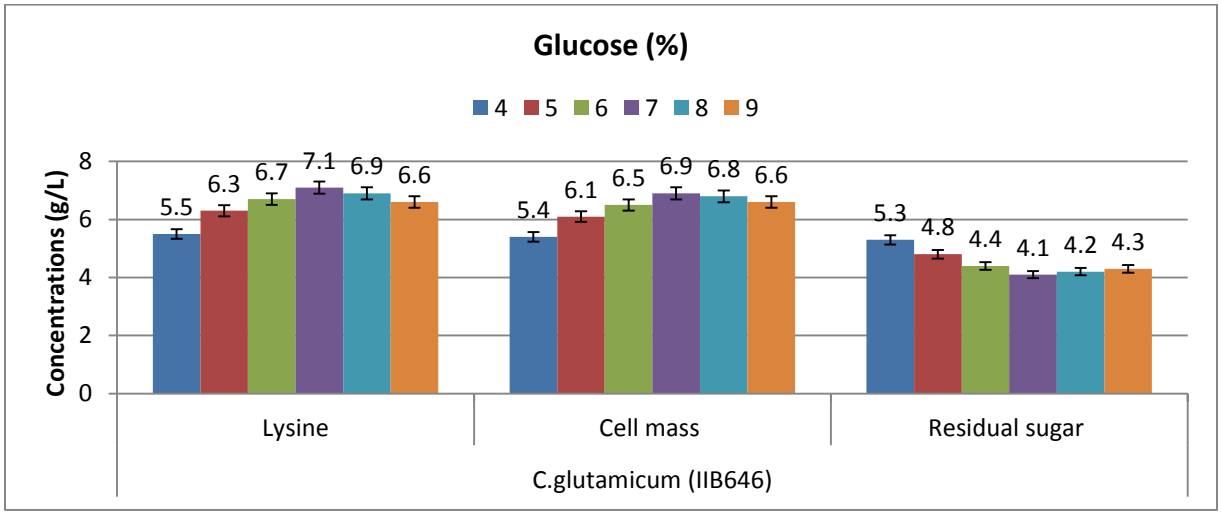

Fig. 5. Optimization of Glucose for Lysine production.

Ferreria and Durate (1991) worked on lysine production and proved that 7 to 10% of glucose amount was optimum for the L-lysine amount enhancement. Residual sugar amount during Lysine production was also investigated by Hirose and Shibai (1985) and it was concluded that higher the concentration of glucose in fermentation media, may inhibit rate of the respective microbial growth along with decrease in synthesis of Lysine and cell masses. While in the low concentrations of carbon sources also caused low Lysine product yield and bio cell masses.

Including yeast extract, peptone, ammonium sulphate, corn steep liquor, potassium nitrate, and urea. These six different nitrogen sources in the concentration of 2% each were screened. Highest 7.0 g/L of Lysine was producing in the existence of ammonium sulphate in the medium M13. In other nitrogen sources, peptone yield 4.3g/l, yeast extract 4.2 g/L, urea 3.1 g/L, corn steep liquor 5.8 g/L and potassium nitrate yield 3.8 g/L of L-lysine (Fig. 6). Further different amounts of ammonium sulphate were also optimized and highest 7.5 g of Lysine was synthesized by M13 medium when it was supplemented with 2.5% ammonium sulphate (Fig. 7).

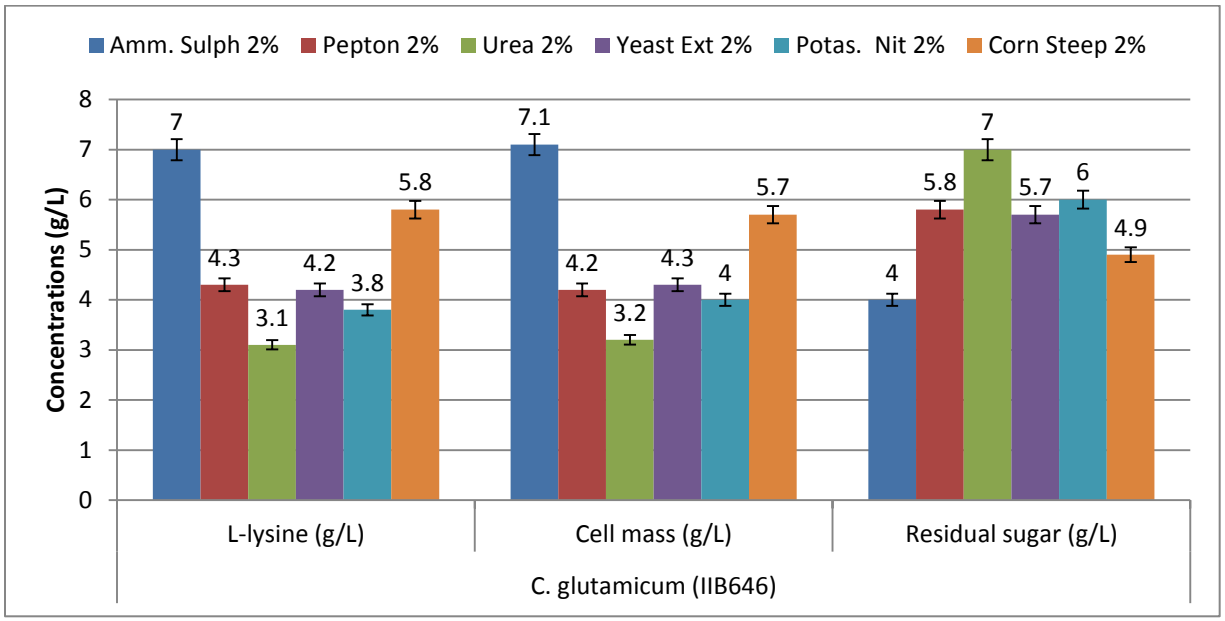

Fig. 6. Screening of nitrogen sources during Lysine production by *C. glutamicum* IIB646.

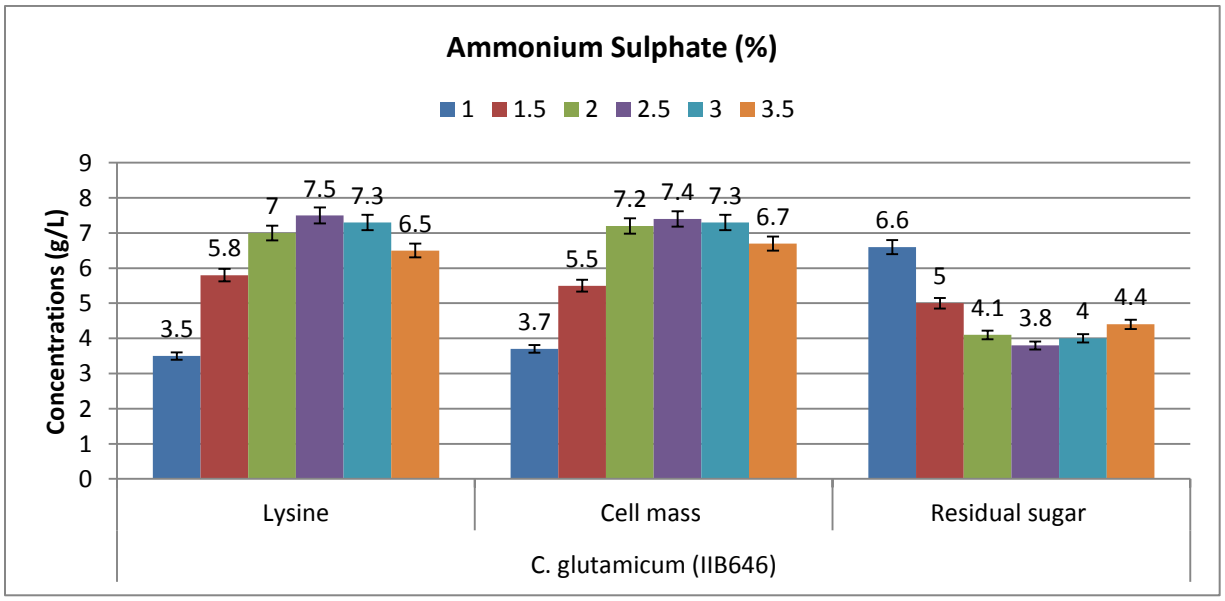

Fig. 7. Optimization of ammonium sulphate for Lysine production by *C. glutamicum* IIB646.

Appropriate source of nitrogen is important for Lysine production. Higher concentration of ammonium is harmful for microbial growth and Less amount of ammonium resulted decrease in product yield so it must be kept at suitable levels. Our results showed good accordance with the study of Shah *et al*. (2002) and Zaki *et al*. (1982) in that study they reported highest L-lysine yield in the existence of optimal amm. sulphate concentrations. Similarly Ferreira and Durate (1991); Hsiao and Glatz (1996) and Wang *et al*. (1991) also suggested amm. sulphate for highest L-lysine yield.

CONCLUSION

From present study, it is concluded that local isolated bacterial culture of *Corynebacterium glutamicum* IIB646 synthesized considerably high concentration of L-lysine in shake flask technique. Lysine production and growth of *Corynebacterium glutamicum* was substantially increased by optimization of cultural conditions and media components. Maximum amount of 7.5 g/L Lysine was synthesized by *Corynebacterium glutamicum* IIB646 in optimized fermentation medium.

REFERENCES

Adnan, A., Z. Mushtaq, Q. Syed and K. Shabbiri (2011).Optimization of fermentation parameters for the production of L-lysine from *Brevibacterium linens* DSM 20158, using statistical approach. *World Appl. Sci. J.*, 13(5): 1132-1140.

Anastassiadis, S. (2007). L- Lysine fermentation. *Recent Pat. Biotechnol*., 1(1): 11-24.

Araki, K., F. Kato, Y. Aral and K. Nakayama (1974). Histidine production by auxotrophic histidine analog-resistant mutants of *Corynebacterium glutamicum*. *Agric. Biol. Chem.*, 38: 837.

Bathe, B., Reynen and C. Pfefferle (2004). L-lysine fermentation. WO04013340A2.

Bercovici D. and M. F. Fuller (2007). Industrial amino acids in nonruminant animal nutrition. In: Wallace RJ, Chesson A (eds) *Biotechnology in animal feeds and animal feeding*. Wiley, Weinheim. doi:10. 1002/9783527615353.ch6.

Broer, S., L. Eggeling and R. Kramer (1993). Strains of *Corynebacterium glutamicum* with different Lysine productivities may have different Lysine excretion systems. *App. Env. Microbiol*., 59(1): 316-321.

Calik, G., F. Unlutabak and T. H.Ozdamar (2001). Product and by-product distributions in glutamic acid fermentation by *Brevibacterium flavum* and effects of the oxygen transfer. *Biochem. Eng. J.*, 9: 91–101.

Cerning, J., C. M. Renard, J. F. Thibault, C. Boullanne, M. Landon, M. Desmazeaud and L. Topisirovic (1994). Carbon source requirements for exopolysaccharide production by *Lactobacillus casei* CG11 and partial structure analysis of the polymer. *Appl. Environ. Microbiol*., 60(11): 3914-3919.

Coello, N., E. Montiel, M. Concepcion and P. Christen (2002). Optimization of a culture medium containing fish silage for L-lysine production by *Corynebacterium glutamicum. Bioresour. Technol*., 85: 207-211.

Cocaign-Bousquet, M., C. Monnet and N. D. Lindley (1993). Batch kinetics in *Corynebacterium glutamicum* during growth on various carbon substrates: use of substrate mixtures to localize metabolic bottlenecks. *Appl. Microbiol. Biotechnol*., 40: 526-530.

Eggeling, L. (2007). *L-Serine and Glycine. In: Amino acid biosynthesis pathway, regulation and metabolic engineering*. Springer, Berlin, vol 5, pp 259–272.

Eggeling, L. (1994). Biology of L-lysine overproduction by *Corynebacterium glutamicum*. *Amino Acids*, 6: 261 – 272.

Fechter, W. L., J. H, Dienst and J. F. Le Patourel (1997). *US Patent No. 5684190.*

Ferreira, C. and C. Durate (1991). Glucose utilization by lysing producing fluroacetate sensitive mutants of *Corynebacterium glutamicum*. *Appl. Biochem*., 27: 251- 257.

Guillout, S., A. A. Rodal, P. A. Lessard and A. J. Sinskey (2002). Methods for producing L-isoleucine. USA patent (US 6451564).

Gubler, M., M. Jetten, S. H. Lee and A. J. Sinskey (1994). Cloning of the pyruvate kinase Gene pyk) of *Corynebacterium glutamicum* and site-specific inactivation of *pyk* in a L-lysine Producing *Corynebacterium lactofermentum* strain. *Appl. Envron. Mirobiol*., 60(7): 2494-2500.

Hirose, Y. and H. Shibai (1985). L-Glutamic acid fermentation. *Comprehensive Biotechnology*, 3: 595–600.

Hsiao, T.Y. and C.E. Glatz (1996). Water reuse in the lysine fermentation process. *Biotechnol. and Bioeng*., 49: 341-342.

Hsieh, C. L., K. P. Hsiung and J. C. Su (1995). Determination L-lysine with ninhydrin ferric reagent. *Anal. Biochem*., 224: 187-189.

Ikeda, M. and R. Katsumata (1992). Metabolic engineering to produce tyrosine or phenylalanine in a tryptophan producing *Corynebacterium glutamicum* strain. *Appl Environ Microbiol*. 58:781–785.

Ikeda, M. (2003). Amino acid production processes. *Adv. Biochem. Eng. Biotechnol*. 79:1–35.

Ikeda, M. (2006). Towards bacterial strains overproducing L-tryptophan and other aromatics by metabolic engineering. *Appl. Microbiol. Biotechnol.*, 69:615–626.

Ishii, T., H. Miwa and M. Yokomori (1997). Process for producing L-lysine by fermentation. *US Patent No. 5650304*.

Kase, H. and K. Nakayama (1975). Fermentation production of L-methionine and regulation of L-methionine biosynthesis in *Corynebacterium glutamicum. Agric. Biol. Chem*., 39:153–160.

Kinoshita, S., S. Udaka and M. Shimono (1957). Amino acid fermentation. Production of L-glutamic acid by various microorganism. *J. Gen. Appl. Microbiol*. 3:193–205.

Kiefer, P., E. Heinzle and C. Wittmann (2002). Influence of glucose, fructose and sucrose as carbon sources on kinetics and stoichiometry of lysine production by *Corynebacterium glutamicum*. *J. Ind. Microbiol. Biotechnol*., 28(6): 338-343.

Leuchtenberger, W., K. Huthmacher and K. Drauz (2005). Biotechnological production of amino acids and derivatives: current status and prospects. *Appl. Microbiol. Biotechnol.*, 69(1):1–8.

Miller, G. L. (1959). Use of Dinitrosalicylic acid reagent for determination of reducing sugar. *Anal. Chem.*, 31(3): 426-428.

Nasri, M., A. Dhouib, F. Zorgaumi, F. Krria and R. Ellouz (1989). Production of L-lysine by using immobilized living C*orynebacterium sp*. *Cell Biotechnol*., 11(2): 865-867.

Nelofer, R., Q. Syed and M. Nadeem (2007). Statistical optimization of process variables for L-lysine production by *Corynebacterium glutamicum*. *Turk. J. Biocehm*., 33: 50-57.

Oh, J. W., S. J. Kim, Y. J. Cho, N. H. Park and J. H. Lee (1993). L-lysine fermentation: *US Patent No. 5268293.*

Pfefferle, W., B.Moeckel, B. Bathe and A. Marx (2003). Biotechnological manufacture of L-lysine. In: Scheper T (ed) *Advances in biochemical engineering*, vol 79. Springer, Berlin, p 59.

Rattray, F. P. and P. F. Fox (1997). Purification and characterization of an intracellular esterase from *Brevibacterium linens* ATCC 9174. *Int. Dairy J.*, 7(4): 273-278.

Rehman, H. U., A. Hameed and S. Ahmed (2012). Selection and characterization of a Lysine yielding mutant of *Corynebacterium glutamicum* - a soil isolate from Pakistan. *Pak. Vet. J.*, 32(1): 20-24.

Ruklisha, M., L. Paegle and I. Denina (2007). L-Valine biosynthesis during batch and fed batch cultivations of *Corynebacterium glutamicum*: relationship between changes and bacterial growth rate and intracellular metabolism. *Progr. Biochem*., 42: 634–640.

Sassi, A. H., A. M. Deschamps and J. M. Lebeault (1996). Process analysis of L-lysine fermentation with *Corynebacterium glutamicum* under different oxygen and carbon dioxide supplies and redox potentials. *Process Biochem.*, 31(5): 493-497.

Shah, H. A., A. Hameed, S. Ahmed and G. M. Khan (2002). Optimization of culture conditions for L-lysine fermentation by *Corynebacterium glutamicum. J. Biol. Sci*., 2(3): 151-156.

Shah, A. H., A. Hameed, S. Rehman and J. Hamid (2012). Nutritional and mutational aspects of L-lysine production by *C. glutamicum* auxotrophs. *Pakistan. J. Zool*., 44 (1): 141-149.

Udaka, S. (1960). Screening methods for microorganisms accumulating metabolites and its use in the isolation of Micrococcus glutamicus. *J. Bacteriol*. 79:754

Wang, J. S., W. L. Chang and T. Y. Liu (1991). Manufacturing of lysine by *Brevibacterium sp*. in a lab scale fermenter. *Chem. Abs.*, 121: 108-120.

Zaki, D., O. Glal, S. A. Hazino-Wabana, I. Morsi and E. A. Wakell (1982). Nutritional report international. *J. Bio. Sci*., 26: 537-546.

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