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Biosynthesis of Lysine in Stirred Fermenter by Corynebacterium Glutamicum

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The amino acids fall among the class of molecules which contain both amino and carboxyl functional group. As the amino acids have both the active groups of an amine and a carboxylic acid they can be considered both acid and base. Amino acids can be produced by four different methods,

a. Extraction b. Synthesis c. Enzymatic catalysis d. Fermentation

Fermentation is the most efficient and widely used technique for the production of amino acids. During fermentation microorganisms use cheap raw materials as fermentation substrates. Amino acids produced by microbial process are generally L-form.

Different microorganisms have been reported to produce different amino acids. Corynebacterium glutamicum is a Gram-positive, non pathogenic and fast growing soil bacterium with special biotechnological importance. It was discovered in the 1950s in Japan as natural producer of glutamic acid and lysine. In the meantime, fermentation processes for C. glutamicum and closely related organisms have been developed for the production of most of the biogene amino acids, but also for further substances such as nucleotides and vitamins.

Aims and Objectives

The objective of the present study was to develop a scheme for the production of Lysine through fermentation by mutants developed from Corynebacterium glutamicum and report its concentration in a view to make feasibility for commercial production of Lysine in Pakistan.

The main objectives of this work were:-

1. Isolation and screening of potent microbial cultures for amino acid production
2. Wild strains optimization for amino acid production.
3. Strains improvement in terms of amino acid production by chemical, UV and combined mutagenesis.
4. To evaluate the potential of molasses and wheat bran as cheap and effective substrate for essential and non-essential amino acid production.
5. Fermentation type and condition optimizations for production of essential and non-essential amino acids.
6. Isolation of amino acids produced
7. Quantitative estimation of amino acids produced by paper chromatographic and methods spectroscopic.

Experimental

•Isolation of Microorganisms from Soil Samples

•Collection of Soil Samples: Soil samples were collected from different parts of Lahore. Isolation Medium A modified Bouillon medium of composition was used for isolation of Corynebacterium glutamicum.

•Preservation of Microbial Cultures

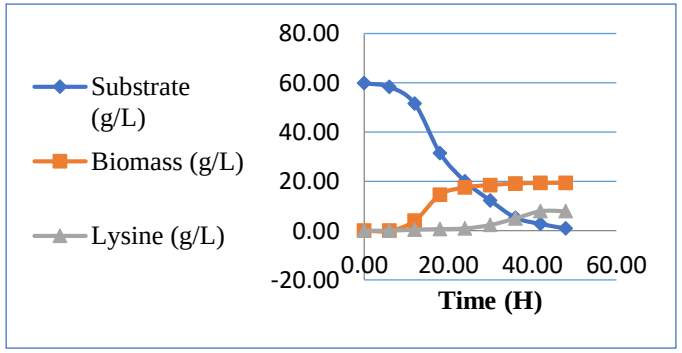
The strain of Corynebacterium glutamicum (C. glutamicum) obtained after identification tests was preserved in 40% and 20% glycerol at -20°C for medium long term storage and in the form of agar slants stored at 4°C

•Fermentation Technique

•Optimization of Fermentation Conditions

Results

L-Lysine were determined in the fermentation broth at different time periods (24, 48, 72 and 96 hours) . The data showed that the growth of Corynebacterium glutamicum increased as the time proceeded (when nutrients are enough) till it reached at maximum level and then started decreasing indicating the depletion of nutrients and accumulation of waste products. Maximum biomass accumulation of 17.4 g/L was observed after 48 hours of fermentation with highest growth rate around 24 hours. However, maximum accumulation of amino acids was observed at 60 hours with maximum rate of production between 45 and 55 hours.



Conclusion

The study provided a cost-effective and well-organized strategy for the production of amino acids. Average biomass yield over a time of 48 hours was 0.337g/g; with a maximum yield within 10 to 20 hours was obtained under optimized conditions: temperature 40°C. The molar yield coefficient of CO_2 was found to be 0.234. The molar yield coefficient of biomass was 0.6 in exponential phase which decreased and fell to near zero at 48 hrs. After 40 hours of fermentation, culture entered into stationary phase, the growth yield turned out to be zero and the total molar yield coefficient of lysine was elevated. Culture conditions of mutants were optimized. Mutants showed optimum lysine production (6.34 g/l) after 48 hrs of fermentation at 34°C with pH 7.3, inoculum size 6 % (v/v) and agitation 140 rpm. Optimization of culture conditions significantly improved amino acid production by mutants.

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