STRAIN IMPROVEMENT AND REACTOR STUDIES FOR THE PRODUCTION OF *L*-METHIONINE BY *CORYNEBACTERIUM LILIUM*

by

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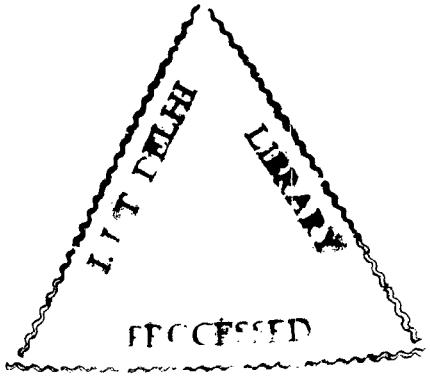

to

my parents
&
my wife

CERTIFICATE

This is to certify that the thesis entitled "**Strain Improvement and Reactor Studies for the Production of L-methionine by *Corynebacterium lilium***" being submitted by **Sanjay Sharma** to the Indian Institute of Technology Delhi, for the award of degree of **Doctor of Philosophy**, has been prepared under my supervision and guidance in conformity with the rules and regulations of the Indian Institute of Technology Delhi. The research report and the results presented in this thesis have not been submitted to any other University or Institute for the award of any degree or diploma.

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ABSTRACT

Methionine is an essential amino acid that is not synthesised by the body and must be obtained from food. It is one of the sulphur containing amino acid and is important in many body functions. There are several existing methods for the production of *L*-methionine, namely, enzymatic production from hydantoin, chemical synthesis of *DL*-methionine followed by enzymatic resolution, continuous production in immobilised cell or enzyme reactors, and submerged fermentative production by auxotrophic or regulatory mutants. Present study was aimed at enhancing the methionine productivity by strain improvement and by reactor studies under different conditions.

The wild type strain of *C. lilium* were subjected to UV induced mutagenesis and mutants resistant to 4 mg/ml, 6 mg/ml and 8 mg/ml of triazole, 150 μg/ml and 200 μg/ml norleucine and 0.5 mg/ml ethionine were isolated. Out of 130 triazole resistant mutants resistant to 4 mg/ml triazole, only 8 produced more methionine than the wild type strain while none of the mutants was found to be stable for 6 mg/ml and 8 mg/ml triazole. Among these 8 mutants *C. lilium* TR-49 was the highest methionine producer giving 60 μg/ml in shake flasks. This is an 8 fold increase over the wild type which produced 7.5 μg/ml in shake flasks. Further, a total of 120 mutants resistant to 200 μg/ml norleucine were isolated. The best among these, *C. lilium* NL-87, produced 372 μg/ml methionine in shake flasks. The methionine yield was 0.068 g methionine/g glucose and specific methionine production was 0.237 mg/g DCW while *C. lilium*

TR-49 mutant resistant to 4 mg/ml triazole produced 60 μg/ml with methionine yield at 0.011 g methionine/g glucose.

UV induced mutagenesis was performed with NL-87 strain to obtain mutants resistant to 200 μg/ml norleucine and 4 mg/ml triazole. A total of 140 mutants were isolated and five of these were over-producers including the *C. lilium* NT-33 and NT-123 strains. In shake flasks *C. lilium* NT-33 produced 521 μg/ml methionine in screening medium, while NT-123 produced 600 μg/ml methionine. The double analogue resistant mutant strain NT-123 was used as the parent strain for the development of triple analogue resistant mutants. A total of 231 mutants resistant to 100 μg/ml norleucine, 4 mg/ml triazole and 0.5 mg/ml ethionine were isolated and 5 mutants were found to be stable with higher methionine productivity. *C. lilium* NTE-99 produced 1.848 g/l methionine after 24 h. The methionine yield was 0.248 g methionine/g glucose and its specific productivity was 1.04 g methionine/g DCW.

Effect of dilution rate and dissolved oxygen on growth and methionine production was studied by the continuous studies of NT-33 strain. The steady state results show higher methionine concentration in the medium at lower dilution rates provided adequate oxygen is present. At a 0.04 h^{-1} dilution rate, a maximum methionine concentration of 1700 μg/ml was obtained at 30% *DO* but the productivity was only 68.02 mg/l·h. In terms of productivity, the maximum for both methionine and biomass occurred at the same dilution rate of 0.17 h^{-1} but at a *DO* level of 58.2% for methionine and 30 % for biomass. These maximum values are 166.35 mg/l·h for methionine and 0.546 g/l·h for biomass.

To study the product formation kinetics in methionine over-producing strain, continuous reactor studies were done with NTE-99 strain. Maximum methionine production at 0.08 dilution rate at 3728 μg/ml. Maximum biomass was also observed at 0.08 dilution rate, but methionine as well as biomass productivities were lower as compared to 0.16 dilution rate where maximum productivities were observed at 0.351 g/l·h and 0.45 g/l·h respectively. A series of fed-batch studies were then carried out to improve upon this result. The highest methionine concentration of 4.074 g/l was obtained by using a feeding medium containing all nutrients of the optimised minimal medium.

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