



Studies on Biochemical Changes during L-Lysine Production in a Synthetic Medium by a Biotin Auxotrophic Mutant *Micrococcus glutamicus* AB₂₀₀

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ABSTRACT

An experimental study was carried out to examine the biochemical changes in the fermentation broth during L-lysine fermentation by the biotin auxotrophic mutant *Micrococcus glutamicus* AB₂₀₀. The importance of this experiment is to study the rate of consumption of carbon and nitrogen source with respect to the rate of formation of the product L-lysine. Production of L-lysine was increased up to 72 hours and then declined, pH increases up to 8.5, dry cell weight showed continuous increased pattern with increased consumption of glucose. Amino nitrogen increases up to 72 hours, then decreased slightly, cell nitrogen and ammonia nitrogen increased continuously where as residual nitrogen decreased sharply.

Keywords: L-lysine; Production; Biochemical changes; Auxotrophs; *Micrococcus glutamicus* AB₂₀₀.

INTRODUCTION

Growth kinetics of microorganism depends on the composition of the nutrient medium, physical parameters of fermentation and the type of organism under investigation. The rate of utilization of carbon and nitrogen source by an organism could be calculated from the estimation of these compounds in the media at different stage of growth. So the studies on the biochemical changes occurring in the media during the fermentation process is essential before going to standardise the large scale production process. *Micrococcus glutamicus*, gram positive bacteria used in industrial production of different amino acids can utilize different carbon and nitrogen source such as glucose, sucrose, different ammonium salts, urea etc.^{1,2} Carbon and nitrogen are important components of cell which plays significant role in cellular constitution, metabolism and reproduction of the organism. The nitrogen source used in the culturing the microorganism is utilized in synthesis of various cellular material like amino acids, proteins, enzymes, hormones, etc. 3% ammonium sulphate and 8% glucose were found to be the most suitable nitrogen and carbon source for *Micrococcus glutamicus* AB₂₀₀ during media optimization process. During the fermentation process, the change in the pH of the media occurs due to production of ammonia & amino acid or lysine.

MATERIALS AND METHODS

Microorganism

A biotin-auxotrophic mutant *Micrococcus glutamicus* AB₂₀₀ developed in our laboratory from a regulatory mutant *Micrococcus glutamicus* AB₅ by induced mutation was used for this study.³

Composition of synthetic medium for L-lysine fermentation

Composition of the synthetic medium used for L-lysine fermentation by the mutant *Micrococcus glutamicus* AB₂₀₀ was as follows: Glucose: 8%; (NH₄)₂SO₄ : 3%; K₂HPO₄:0.2%; MgSO₄.7H₂O: 0.25%; CaCO₃: 0.05%; FeSO₄.7H₂O: 10 µg/mL; ZnSO₄.7H₂O: 5 µg/mL; MnSO₄.H₂O: 5 µg/mL; Biotin: 1 µg/mL; pH : 7.2.

Physical conditions for the fermentation

The fermentation was carried out using the shake flask method on a rotary shaker (150 rpm) in 100 ml Erlenmeyer conical flask containing 25 ml synthetic medium, for 72h at 30°C. The medium was inoculated with 4.0% (v/v) of 48h old seed culture (7.4 x 10⁷ cells/mL) of *Micrococcus glutamicus* AB₂₀₀.³

Determination of pH

pH of the medium was determined with the aid of a previously standardized pH meter (Unicam 9450 model).

Estimation of residual sugar

Residual sugar was determined by Nelson & Somogyi method.⁴

Estimation of Nitrogen

Total nitrogen in the cell and in the broth was estimated by micro Kjeldahl's method⁵, ammonia nitrogen was estimated by the method of Conway⁶ and amino nitrogen calorimetrically⁷.

Estimation of Dry Cell weight (DCW)

Dry Cell weight was estimated by the method as proposed by Shah et al.⁸

Analysis of Amino acid

Qualitative estimation of L-lysine was done by descending paper chromatography. Ninhydrin method was employed for quantitative estimation of L-lysine.⁹

RESULTS AND DISCUSSION

Fig 1 shows the pattern of change of pH, residual sugar concentration, and dry cell weight & lysine concentration at different fermentation period. Initial pH of the fermentation medium was 7.2 (which were optimized during the optimization of physical parameters)³ with increase in lysine production, the pH increases up to 8.49

at 72 h of fermentation with increase in lysine production up to 22.21mg/mL. After that pH and lysine concentration decreases slightly up to 120 hour. Dry cell weight increases up to 120 h of fermentation along with decreases in residual glucose concentration. After 72 hour the glucose consumed was utilized for increasing biomass but not the lysine production.

Chio et al., showed similar pattern of residual sugar concentration, dry cell weight and L-glutamic acid concentration change.¹⁰ Lee et al., studied the pattern of dry cell weight changed along with glucose concentration during L-threonine production.¹¹

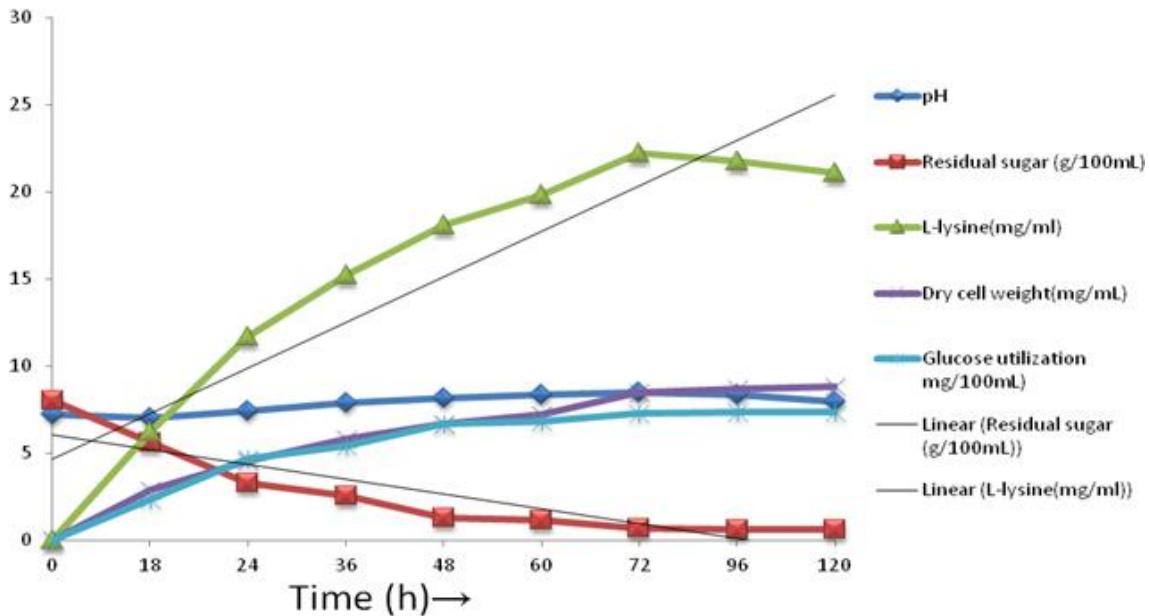


Figure 1: Rate of production of L-lysine in relation to pH & glucose concentration (Values expressed as mean ±SEM, where n=6)

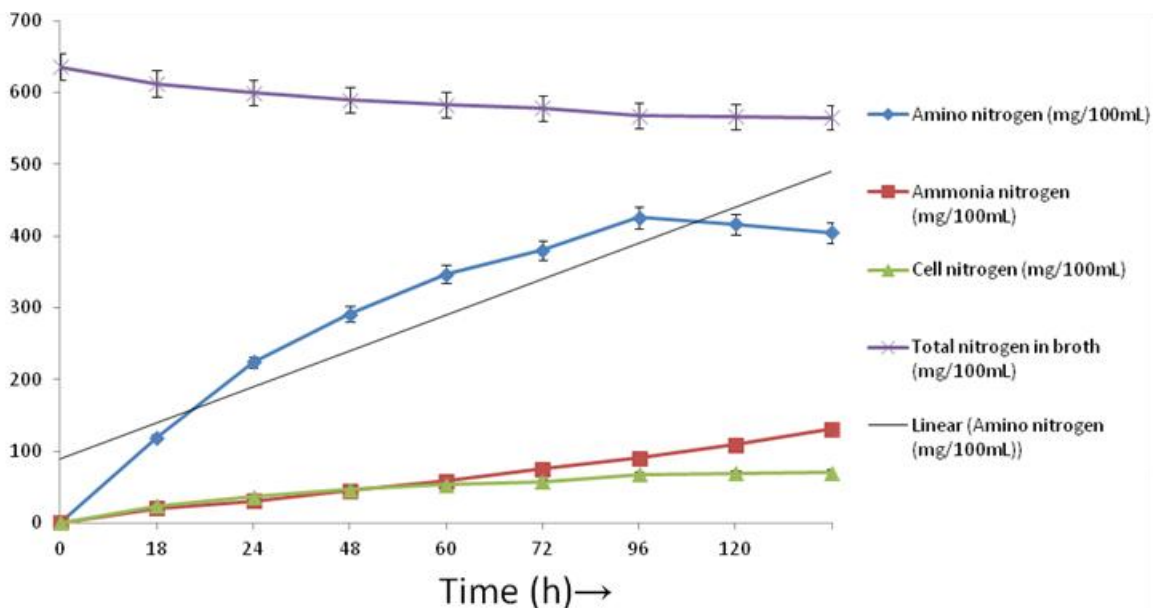


Figure 2: Rate of production of L-lysine in relation to amino nitrogen, ammonia nitrogen, cell nitrogen & total nitrogen in broth

Fig: 2 showed the pattern of different form of nitrogen changes during different intervals of fermentation. Carito and Pisano also reported similar pattern of different form of nitrogen changes during production of L-alanine.¹² Amino nitrogen which corresponds to the product nitrogen was calculated by estimating L-lysine at regular intervals of time, showed increasing pattern up to 72 h of fermentation and decreased slightly up to 120 h. 3% ammonium sulphate was the best nitrogen source selected during the study of optimization of nutrients. Ammonia nitrogen increases throughout the study. Cell nitrogen also increases up to 120 h but total nitrogen in the broth (= amino N + ammonia N+ residual N in broth) was decreased constantly. So out of the total nitrogen source, a part was utilized for lysine production, a part for increasing the biomass, another part evolved as ammonia and rest of the nitrogen source was in the fermentation broth as unutilized nitrogen.

CONCLUSION

94.5 % nitrogen source and 96 % carbon source was utilised by the mutant *Micrococcus glutamicus* AB₂₀₀ during the fermentation process. 22.21g/L L-lysine was formed with 8 % glucose & 3 % ammonium sulphate as the best carbon and nitrogen source.

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