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Optimization of Glutamic Acid Production by *Brevibacterium roseum**

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Abstract: The production of L-Glutamic acid in submerged fermentation of *Brevibacterium roseum* has been investigated through several parameters. All the nutritional ingredients in the production medium were optimized individually and production condition parameters like incubation time, temperature, pH was determined. Various carbon and nitrogen sources along with Biotin at different concentrations have been added to the culture medium, in order to assess their efficiency in L-Glutamic acid production. The maximum L-Glutamic acid obtained is 40.5 mg mL^{-1} in 96 h with pH 6.0 and 150 rpm of agitation.

Key words: L-Glutamic acid, submerged fermentation, *Brevibacterium roseum*, optimization

Introduction

L-Amino acids have a wide spectrum of commercial use of food additives, feed supplements, infusion compounds, therapeutic agents and precursors for the synthesis of peptides or agrochemicals. Mono Sodium Glutamate (MSG), the sodium salts of glutamic acid is used commercially as a flavor enhancer, usually in combination with nucleotides inosinate to provide an expansion and extension of taste in processed food such as soups, biscuits, noodles, Chinese foods, meat and vegetable processing etc. (Kinoshita, 1985; Hirose *et al*., 1985). Glutamic acid mother liquor in MSG production is being used in the manufacture of Sauce and as soil conditioner, fertilizer etc. Several strains of *Brevibacterium* and *Corynebacterium* are used as cost effective bioconverters, which have been exploited by the fermentation in the history to provide various amino acids, including L-glutamic acid (Kumagai, 2000; Ikeda, 2003). Owing to the importance of particular industrial fermentation, much efforts were still going on to improve the glutamic acid fermentation process especially from the standpoint of savings and production cost (Das *et al*., 1995; Nampoothiri and Pandey, 1995b). The present study was intended to standardize the various optimal parameters for a strain of *Brevibacterium roseum* for glutamic acid production and also studied the effect of various carbon nitrogen and different biotin concentrations on glutamic acid yield.

Materials and Methods

Organism

Brevibacterium roseum NCIM 2270 obtained from NCL, Pune, India was used for the study and was maintained on Nutrient Agar medium. Sub culturing was carried out once in a every three weeks and the culture was stored at 4°C.

Medium for Glutamic Acid Production

Production medium contain glucose 5.0% w/v, Urea 0.8% w/v, Dipotassium hydrogen phosphate 10% w/v, Magnesium sulphate 2.5% w/v, Manganese sulphate 0.1% w/v, Ferrous sulphate 0.1% w/v,

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d-Biotin 2 μg L^{-1}, Calcium carbonate 1.6% w/v, Calcium chloride 0.1 M in distilled water (pH 7.2). The medium was autoclaved at 120°C, 15 psi for 20 min. Glucose and Urea were sterilized separated by microfiltration.

Inoculum Preparation

Inocula were prepared from the freshly grown culture as described by Nampoothiri and Pandey, 1995. 10 milliliter of sterile water was transferred to a culture [5 days old]. From the nutrient agar slant culture, the culture was dislodged using the inoculation needle. 5 milliliter of the spore suspension was transferred into 250 mL Erlenmeyer flask containing 50.0 mL of sterile inoculum medium. The composition of inoculum medium was Sodium Chloride 0.5% w/v, Peptone 1.0% w/v, Beef Extract 1.0% w/v in distilled water. The cells were cultivated in this medium at 30°C on a rotary shaker at 150 rpm for 72 h.

Submerged Fermentation

The *Brevibacterium roseum* was cultured on production medium by submerged fermentation. To each flask, the inoculum of a 24 h old culture was added and incubated in a rotary flask shaker. After incubation, the samples were filtered and centrifuged to remove the cell particles and other residues and glutamic acid in the broth was estimated.

Analytical Methods

Thin layer chromatography (silica gel G, solvent mixture: n-butanol /glacial acetic acid/water 4:1:1 v/v) was used for the qualitative determination of L-glutamic acid (Brenner and Niederwiser, 1967) and it was estimated quantitatively by a ninhydrin colour reaction (Spies, 1957). Soluble sugars were estimated by the dinitro salicylic acid (DNS) method (Miller, 1959).

The present investigation has been carried out at Center for Biotechnology, Department of Chemical Engineering Andhra University, A.P., India in the year 2004.

Results and Discussion

The effect of pH on glutamic acid was determined among the 5 levels of initial pH of the medium was used, the glutamic acid concentration was higher at pH value of 6.0 (Fig. 1). A fermentation time of 96 h was found to be favorable for maximum glutamic acid yield (Fig. 2). To determine the optimum incubation temperature, flasks with production medium were incubated at different temperatures ranging from 20°C to 34°C. The optimum temperature was found to be at 30°C with 27.8 mg mL^{-1}

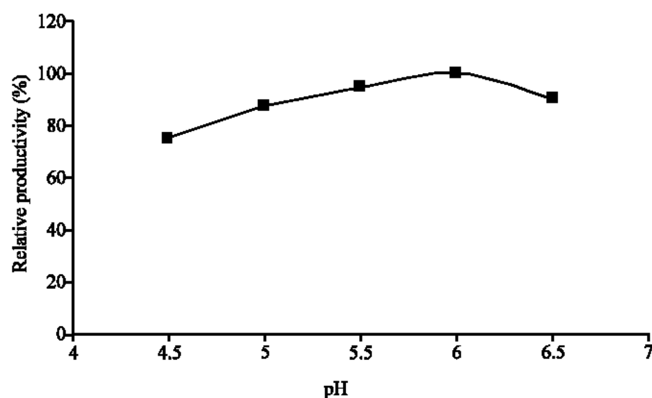

Fig. 1: Effect of pH on glutamic acid production

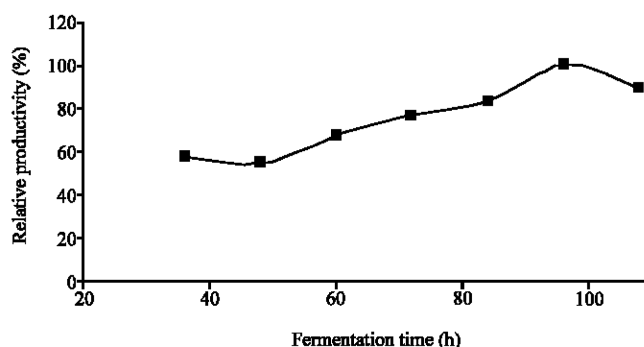

Fig. 2: Effect of fermentation time on glutamic acid production

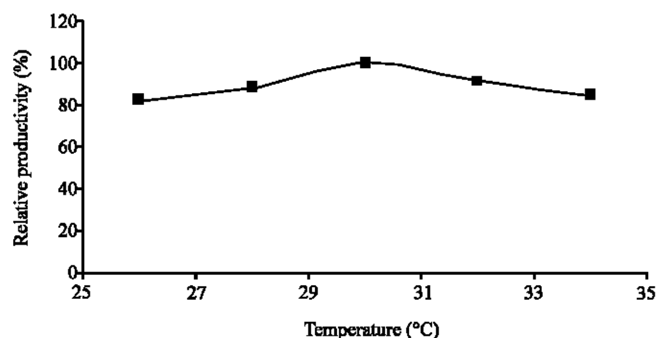

Fig. 3: Effect of temperature on glutamic acid production

of glutamic acid. At the lower level and at 34°C the glutamic acid yield in the medium was considerably lower as shown in the (Fig. 3). Among five different Biotin concentrations used, the optimum to be 3.0 μg mL^{-1} with 35.5 mg mL^{-1} of glutamic acid. The change of agitation did not affect the glutamic acid but only a marginal increase was observed with 150 rpm. The estimation of reducing sugars at periodic intervals in broth during fermentation showed that the consumption of glucose increase with the time of fermentation and about 80-85% of glucose was consumed after 96 h. Each ingredient of the production medium was optimized. The glutamic acid yields were significantly increased with change of each ingredient concentration (Table 1). Clearly shows that the glutamic acid concentration can be increased from 27.6 mg mL^{-1} with basal production medium to 40.5 mg mL^{-1} with optimization of all nutrients. It was difficult to evaluate that which ingredient has maximum effect on glutamic acid, since the optimum process was carried out step by step.

Analysis of variance showed that there was a significant improvement in the glutamic acid content, which was observed with different carbon and nitrogen sources. The supplementation of ethanol, lactose and maltose were the best suitable carbon sources with 52.7, 48.8 and 47.2 mg mL^{-1}, respectively for glutamic acid production (Table 2). Five different nitrogen sources were used as supplementation for production medium. Ammonium phosphate (54.1 mg mL^{-1}), liquid ammonia (50.8 mg mL^{-1}) and urea (49.2 mg mL^{-1}) have significantly influenced the glutamic acid yield (Table 3). The glutamic acid and the production conditions observed in the present study are comparable with earlier research findings. Jyothi *et al.* (2005) found that the temperature of 30°C and pH 7.0 were optimum for glutamic acid production with *Brevibacterium divaricatum*. They also observed that ammonium nitrate and urea as nitrogen supplements improved the glutamic acid yield.

Table 1: Optimization of nutrient ingredients concentration for glutamic acid production with *Brevibacterium roseum*

	Nutrient concentration used with basal production medium		Effect of nutrient ingredient concentration on glutamic acid	
Nutrient ingredient	Conc. used	Glutamic yield	Nutrient optimized	Glutamic yield (mg mL^{-1})
Glucose	5.0 % w/v	27.6	8.0 % w/v	33.8
Biotin	2.0 μg mL^{-1}	27.6	3 μg mL^{-1}	35.1
Urea	0.8 % w/v	27.6	0.8% w/v	34.8
Calcium chloride	0.1 M	27.6	0.3 M	35.5
Calcium carbonate	1.6 % w/v	27.6	1.8% w/v	35.0
Dipotassium hydrogen sulphate	0.1 % w/v	27.6	12.5% w/v	38.8
Magnesium sulphate	0.025% w/v	27.6	2.5% w/v	38.0
Manganese sulphate	0.001% w/v	27.6	0.3% w/v	40.3
Ferrous sulphate	0.001% w/v	27.6	0.3% w/v	40.5

Table 2: Effect of supplementary additions as carbon sources on glutamic acid production

	B. roseum	
Carbon source (6 % w/v)	GA (mg mL^{-1})	Conversion rate
Glucose	33.8	42.25
Maltose	47.2	59.00
Fructose	40.7	50.87
Lactose	48.8	61.00
Ethanol	52.7	65.87

Table 3: Effect of supplementary additions as nitrogen sources on glutamic acid production

	B. roseum	
Nitrogen source (0.8 % w/v)	GA (mg mL^{-1})	Conversion rate
Ammonium phosphate	54.1	67.62
Ammonium chloride	40.3	50.37
Ammonium sulphate	40.1	50.12
Urea	49.2	51.5
Liquid ammonia	50.8	63.5

Joseph and Rao (1973) reported that the maximum yield of glutamic acid was found with 0.5% urea concentration by *Micrococcus glutamicus*. Nampoothiri and Pandey (1996) observed that the maximum glutamic acid with glucose as a carbon source and urea as a nitrogen source with 48 h of fermentation and about 90% of glucose was consumed by *Brevibacterium* species. The selectivity of the carbon and nitrogen sources by different microbial strains could be the reason for the observed yields (Nampoothiri and Pandey, 1995; Das *et al*., 1995).

Conclusions

The study indicates that comparatively good yields of glutamic acid can be obtained with *Brevibacterium roseum*. The study also proved that the optimization of nutrient concentration has a positive impact on glutamic acid production. Maximum yields of glutamic acid were obtained when the different carbon and nitrogen sources were used as supplements. In the present study only limited carbon and nitrogen sources were used. An attempt to utilize other cheaply available carbon and nitrogen sources by submerged fermentation and the scale up of the process is under way.

Acknowledgments

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