roots suggesting thereby that the comparison of CEC values of different materials will be affected if Crooke's method is used. The method is precise, rapid and it completely eliminates the acid drift, since the titration is carried out in the absence of roots.

TABLE I
Comparison of the method

Plant	*CEC (m.e. per 100 g dry roots)		†Difference (Acid-drift)
	Crooke's method	Method described	
Monocots			
Hybrid napier	5·17	4·99	0·18
Tripsacum lexum	7·49	6·52	0·97
Cocos nucifera	12·17	9·82	2·35
Dicots			
Crotalaria sp.	10·13	9·01	1·12
Vigna sp.	14·72	11·10	3·62
Stylosanthes sp.	23·00	20·10	2·90
Cleome sp.	23·64	21·23	2·41

* Mean of three values.

† Difference is not statisucally significant.

TABLE II
Precision of the method

Plant	Nc. of measure-ments	CEC (m.e. per 100 g dry roots)		
		Range	Mean	SD*
Vigna sp.	8	10·62–11·58	11–16	0·445
Cleome sp.	8	19·78–22·20	21·11	0·928
Crotalaria sp.	8	8·69–10·61	9·17	0·678
Tripsacum lexum	8	5·80– 7·23	6·28	0·413
Hybrid napier	8	4·83– 5·79	5·01	0·334
Cocos nucifera	8	8·69–10·14	9·45	0·443

* Standard deviation.

Grateful acknowledgement is made to Mr. K. V. Ahamed Bavappa, Director, for his interest and encouragement in this study. We wish to thank Miss R. Thankamma for assisting in the work and Mr. M. V. George and Mr. M. P. Abdul Razak for carrying out the statistical analysis of the data.

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EFFECT OF NITROGEN SOURCES ON L-LYSINE PRODUCTION BY HOMOSERINE AUXOTROPH OF *MICROCOCCUS GLUTAMICUS*

ABSTRACT

Of the various nitrogen sources tested ammonium acetate resulted in the maximum production of L-lysine by homoserine auxotroph of *M. glutamicus*. Ammonium sulphate and ammonium chloride were also good, whereas urea, potassium nitrate and ammonium nitrate were not suitable for L-lysine production. The optimum concentration of both ammonium acetate and ammonium sulphate was 2%. The L-lysine fermentation efficiency expressed as g L-lysine produced/100 g glucose consumed was 33% when ammonium sulphate was used and 46% when ammonium acetate was used. The fermentation period was reduced by 12 hr when ammonium acetate was used instead of ammonium sulphate.

IN order to obtain maximum yield of a fermentation product, it is essential to find out optimum cultural conditions. A number of workers have studied the effect of various cultural conditions on the production of amino acids by different microorganisms[1-4]. The effect of different nitrogen sources on the production of L-lysine by *M. glutamicus* (homoserine⁻) is reported in this paper.

Micrococcus glutamicus (homoserine⁻) was maintained on nutrient agar. Seed inoculum was prepared according to Lodha *et al.*[5]. The details of cultivation for L-lysine production have been reported[5]. The basal fermentation medium contained (g/litre): glucose, 80; K_2HPO_4, 0·5; KH_2PO_4, 0·5; $MgSO_4.7H_2O$, 0·25: $FeSO_4.7H_2O$, 0·01; $MnSO_4.4H_2O$, 0·01; $CaCO_3$, 10; DL-threonine, 0·2; L-methionine, 0·2; DL-isoleucine, 0·2 and *d*-biotin, 30 μg. The pH was adjusted to 7·9 and the medium was sterilized at 10 lb pressure for 10 min.

L-lysine accumulated in the culture broth was determined colorimetrically by the acidic ninhydrin method of Chinard[6]. Glucose in the culture broth was determined by Anthrone method as described by Morris[7].

Effect of nitrogen sources.—The results presented in Table I show the effect of different nitrogen sources on L-lysine production. Ammonium acetate was found to be the best nitrogen source followed by ammonium sulphate and ammonium chloride. With ammonium nitrate, potassium nitrate and urea, the amount of lysine produced was only 1·5, 3·0 and 4·5% of the lysine produced with ammonium acetate. With increase in ammonium acetate or ammonium sulphate concentration up to 2%, there was increase in L-lysine production (Table II). Further increase in the concentration of both the nitrogen sources resulted in lower yield of L-lysine,

TABLE I

Effect of different nitrogen sources on L-lysine production

Nitrogen source*	L-lysine produced HCl salt, g/litre
$(NH_4)_2SO_4$	13·3
$(NH_4)_2HPO_4$	6·1
NH_4NO_3	0·3
NH_4Cl	12·2
CH_3COONH_4	19·4
KNO_3	0·6
$(NH_2)_2CO$	0·9

* to give 4·24 g N/litre medium.

TABLE II

Effect of ammonium sulphate and ammonium acetate concentration on L-lysine production

Ammonium sulphate concentration %	L-lysine produced HCl salt, g/litre	Ammonium acetate concentration %	L-lysine produced HCl salt, g/litre
0·5	4·7	1·0	9·3
1·0	10·4	1·5	15·9
1·5	12·5	2·0	22·7
2·0	14·0	2·5	21·6
2·5	13·5	3·0	19·4
3·0	11·8	..	..

Nitrate-nitrogen might be acting as repressor for L-lysine production. The unsuitability of urea could perhaps be due to inhibition by any of the metabolites formed from it. Superiority of ammonium acetate could be due to the presence of acetate ions or due to its buffering action.

Chemical changes during L-lysine fermentation.— Glucose utilization and pH changes during L-lysine fermentation were observed along with the amount of L-lysine produced at different periods using ammonium acetate and ammonium sulphate as nitrogen sources @ 2%. The results are illustrated in Fig. 1.

With both the nitrogen sources the amount of L-lysine produced increased linearly with time upto 60 hr with an initial lag of about 12 hr. However, the optimum fermentation period with ammonium sulphate was found to be 72 hr compared to 60 hr with ammonium acetate. The glucose content of the culture broth decreased linearly until when L-lysine production was maximum. With ammonium acetate more glucose was consumed and this was also reflected in higher production of L-lysine compared to when ammonium sulphate was used. The higher amount of L-lysine produced with ammonium acetate could perhaps be due to its higher buffering action, as seen from the results of pH changes. There was marked decrease in pH (from 7·0 to 5·1) towards acidic range when ammonium sulphate was used, whereas with ammonium acetate pH changed from 7·2 to 8·0.

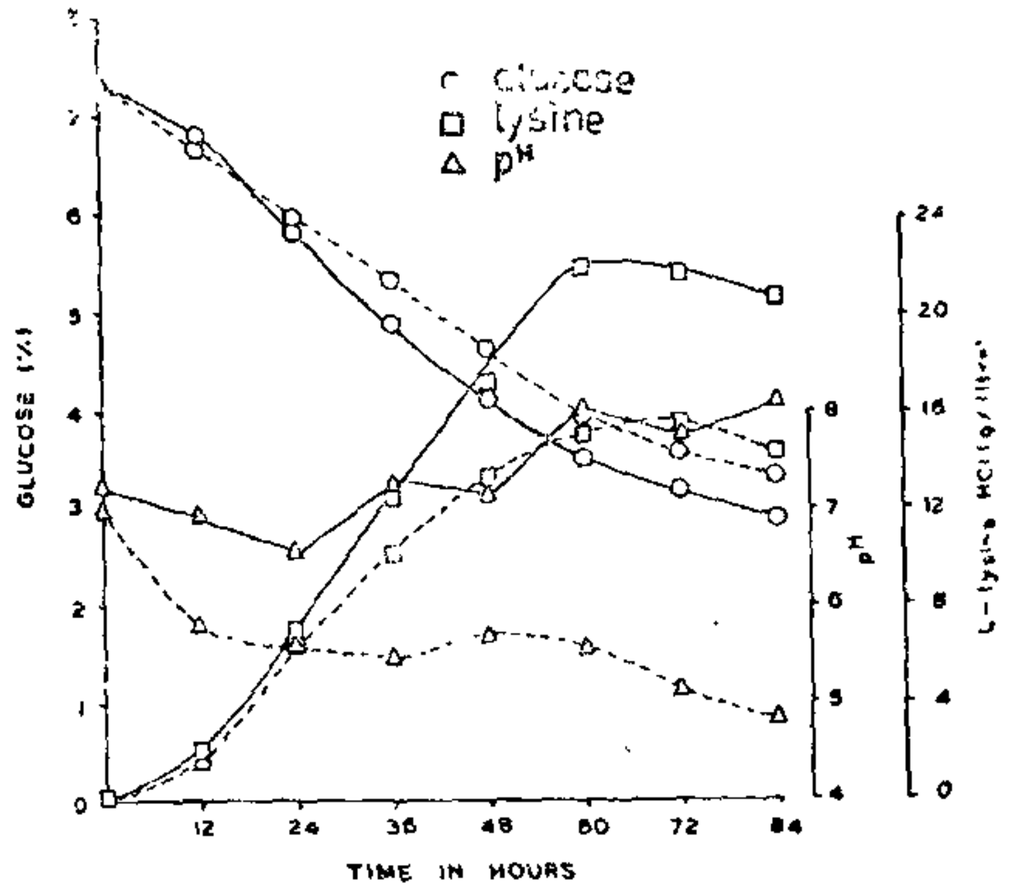

FIG. 1. Chemical changes during lysine fermentation. ———— with ammonium acetate, — — — with ammonium sulphate.

The L-lysine fermentation efficiency expressed as g L-lysine produced/100 g of glucose consumed was found to be 33% when ammonium sulphate was used and 46% when ammonium acetate was used as nitrogen source at the optimum fermentation periods of 72 hr and 60 hr respectively (Fig. 1). Kinoshita *et al.*[8] obtained a fermentation efficiency of only 29% with ammonium chloride at the optimum fermentation period of 72 hr. Therefore, the use of ammonium acetate as a sole source of nitrogen, in the fermentative production of L-lysine, is very promising.

The authors are thankful to Dr. M. S. Naik for his interest and help. One of us (M. L. L.) is grateful to the Indian Council of Agricultural Research for awarding a Senior Research Fellowship.

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