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Production of α-Keto Acids with Alginate-Entrapped Whole Cells of the Yeast *Trigonopsis variabilis*

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Abstract

The yeast, *Trigonopsis variabilis*, was immobilized by entrapment in alginate. The immobilized cells containing high amounts of D-amino acid oxidase were used to convert D-amino acids to their corresponding α-keto acids.

Index Entries: α-Keto acids, from immobilized yeast; immobilized yeast, α-keto acid from; yeast, α-keto acid from immobilized; alginate entrapped yeast; entrapped yeast, α-keto acid from; *Trigonopsis variabilis*, keto acids from immobilized; oxidase, amino acid; amino acids, conversion to α-keto acids.

Introduction

Keto acids may serve as effective nitrogen-deficient nutritive substitutes for many of the essential amino acids. The corresponding essential amino acids are formed in vivo primarily by transamination at the expense of the nonessential amino acids, which are converted to their keto acid analogs in this process. Another process that may result in the conversion of keto acids to amino acids is the direct utilization of ammonia, which is believed to take place in the liver.

By decreasing the intake of nitrogen associated with protein synthesis, keto acid therapy may forestall or decrease the frequency of dialysis treatment for chronically uremic patients. However, the lack of suitable methods of preparation of the

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keto analogs of the essential amino acids has hampered the thorough investigation of such a therapeutical approach.

We have developed a process for the production of keto acids from the corresponding amino acids based on immobilized whole cells of the yeast *Trigonopsis variabilis* (*1*).

Experimental

T. variabilis contains high amounts of D-amino acid oxidase, which catalyzes the following reaction:

$$RCNH_2COOH + O_2 + H_2O \rightarrow RCOCOOH + H_2O_2 + NH_3$$

Cells and manganese oxide were co-entrapped in calcium alginate. The hydrogen peroxide formed was efficiently degraded by the manganese particles and higher local concentrations of oxygen were obtained. Furthermore, an efficient oxygen transfer was achieved by using a trickle-bed reactor.

The enzyme showed broad substrate specificity and the majority of the amino acids tested were converted to the corresponding keto acids with relatively high activity. The K_m values for most substrates are in the range from 0.5 to 2 m*M*. The pH-optimum for the enzyme is 8.0 and the activity is increased with temperature up to about 50°C. Treatment at 60°C for 5 min results in complete loss in activity.

Depending on the object of the production, i.e., simultaneous production of keto acids and L-amino acids from racemic mixtures, or only production of keto acids, the flow rate of substrate through the reactor must be varied (Fig. 1). In the former

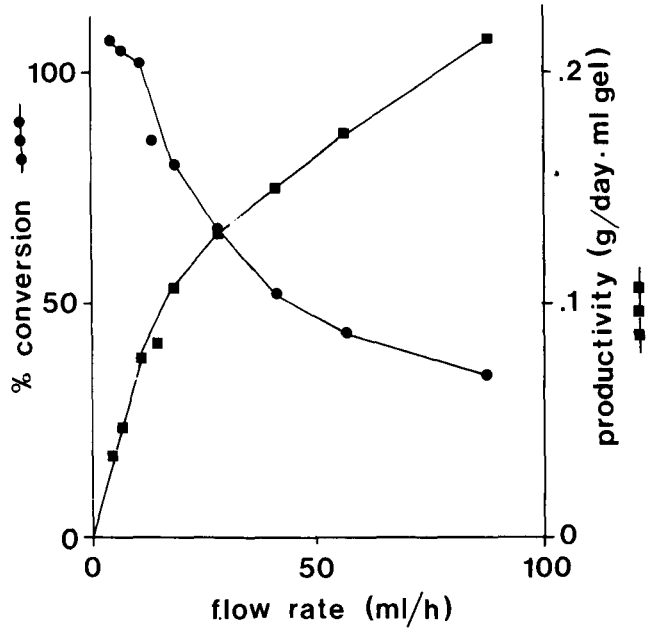

Fig. 1. Effect of flow rate on the conversion of D, L-methionine to γ-keto-α-methiolbutyric acid. The beads contained 20% (w/w) wet cells of *T. variablis*.

case, complete conversion occurs if sufficiently low flow rates are used. In the latter case a compromise must be made since very high flow rates result in large volumes of solution, which could create problems on the refinement of the product.

References

1. Brodelius, P., Hägerdal, B., and Mosbach, K., in Weetall, H. H., and Royer, G. P., eds., *Enzyme Engineering*, **5**, 383 (1980).