RESERVE COPY.

PATENT SPECIFICATION

NO DRAWINGS

849,280

Date of Application and filing Complete Specification: March 20, 1957.

No. 9183/57.

Application made in Japan on Oct. 29, 1956.

Complete Specification Published: Sept. 21, 1960.

Index at acceptance:—Class 2(3), C2B2(A1 : G1A : G8 : K), C3A8(A1 : C4 : K).

International Classification:—C07c.

COMPLETE SPECIFICATION

Method of producing l-Glutamic Acid

We, KYOWA HAKKO KOGYO KABUSHIKI KAISHA, a corporation organised and existing under the laws of Japan, of 9, Yurakucho - 1 - chome, Chiyoda - ku, Tokyo, Japan, do hereby declare the invention for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

This invention relates to a method of producing 1 - glutamic acid in a remarkably high yield by culturing certain strains of microorganisms in a culture medium which contains saccharidic materials, nitrogen sources, inorganic materials, and any other suitable materials, if desired. More specifically the present invention provides a method of improvement for producing 1 - glutamic acid by fermentation.

In Specifications Nos. 8505/57 and 9182/57 (Serial Nos. 839,597 and 849,279) there are disclosed methods of producing and accumulating 1 - glutamic acid, and other amino acids directly in a culture medium by culturing microorganisms which satisfy two biochemically specific requirements.

One of the requirements is the ability of the strain to produce α - ketoglutaric acid (or α - ketoacids, generally) from saccharidic materials, and the other requirement is a strong 1 - glutamic acid (or amino acid generally) dehydrogenase activity of the strain, particularly its strong activity towards reductive amination of α - ketoglutaric acid (or other α - ketoacid) of the above reversible enzymatic reaction. The Specifications also disclose that the fermentation conditions affect the yields to a large extent, and the pH value of the culture medium should always be maintained in the range from 6.0 to 9.0 by suitable fermentation control such as by addition of acid - neutralising agents to the fermenting medium.

In addition to the above - mentioned two biochemical requirements of the strain used and the proper control of pH value of culture medium, it has now been found that it is advantageous if the propagation of microorganism is controlled properly for the increase of the yield of 1 - glutamic acid.

As should be easily understood, such a type of fermentation, as that producing 1 - glutamic acid, consists of various biochemical steps of degradation and synthesis starting from the substrates. A reasonable fermentation control is especially needed to obtain a high yield of the acid. For this purpose, it has been found advantageous to use such a strain that is vitamin - less.

It is well known that there are some strains among microorganisms which require particular vitamins or particular amino acids for their growth, and such strains exhibit, as a rule, a sharp growth response on the amount of vitamins or amino acids given in the culture medium. Consequently, if such vitamin- or amino acid-requiring strains are employed, the propagation of microorganisms and correspondingly the enzymatic activity of a cell population can appropriately be controlled to a requisite level by proper addition of the required vitamins or amino acids. Therefore fermentation progress can advantageously be controlled as desired.

Investigation was directed to the selection of strains with respect to their vitamin requests, and it was found that biotin - requesting strains were suitable for the above-mentioned purposes. There is much to be clarified on the biochemical function of biotin, but it can be presumed that the function of biotin is particularly important in the case of the fermentation producing 1 - glutamic acid in view of the facts reported in the following references:—

Arch. Sci. Physiol. *7*, 85 (1953); J. Sci. Ind. Research, *13B*, 110 (1954); J.B.C. *177*, 125 (1949).
(These reports show that biotin participates in dissociation and synthesis of protein.)

Arch. Biochem, Biophys. *55*, 307 (1955); J.B.C. *212*, 217 (1955); Trans. N.Y. Acad. Sci. *15*, 159 (1953); Physiol. Revs. *21*, 1 (1941).
(These reports show that biotin has close

[*Price 3s. 6d.*]

relations with sugar metabolism particularly with fixation and liberation reactions of carbon dioxide.)

Accordingly, to use a biotin - requesting strain and to control the biotin content in a culture medium provides a highly advantageous fermentation control process in such a biochemically complicated fermentation as that producing 1 - glutamic acid.

According to the invention, therefore, there is provided a method of producing 1 - glutamic acid, comprising culturing in a culture medium a microorganism which has biotin-requesting nature, is capable of producing α - ketoglutaric acid from saccharidic materials, and possesses a high activity of 1 - glutamic acid dehydrogenase, particularly a strong activity for reductive amination of the α - ketoglutaric acid, there being present an amount of biotin in the culture medium so as to maintain a favourable balance between the propagation of the microorganism and ferementation, while maintaining the pH value of the culture medium at 6—9 by addition of an acid - neutralising agent, so that 1 - glutamic acid is produced in the medium in a high yield.

It is well known that biotin is contained in various natural substances, such as egg yolk, kidney, liver, yeast, milk and molasses, and such natural substances and/or their extracts, hydrolysates, and/or digests may be used in place of pure biotin with substantially equivalent results. Consequently, our invention is never limited to the use of pure biotin.

The following Example is given to illustrate the invention.

EXAMPLE 1.

Composition of culture medium by weight.

Glucose	10%
K_2HPO_4	0.1%
$MgSO_4.7H_2O$	0.025%
$FeSO_4.7H_2O$	0.001%
$MnSO_4.4H_2O$	0.001%
Water	ad 100%

Biotin was added to this culture medium in various concentrations. A biotin requiring strain of Micrococcus isolated from soil and named Micrococcus No. 541 was employed for the tests. Micrococcus No. 541 differs from Micrococcus No. 534 given in the previously-mentioned Specification No. 8505/57 in its biotin request only, but has substantially the same morphological, physiological, and other properties as the latter. 1 - Glutamic acid production with various biotin levels were determined by shaking culture. Table I gives the results after three day cultivation at 28° C., the pH value of the culture medium being maintained in the range from 6.5 to 8.5 by intermittent additions of urea.

TABLE I

Biotin Content (γ/l)	l-glutamic acid (mg/ml)	Dried Cell weight (mg/10 ml)
0.5 — 1.0	20.5	10 — 25
1.0 — 2.5	37.5	25 — 60
2.5 — 5.0	17.0	60 — 120
5.0 — 7.5	6.7	120 — 180
7.5 — 10.0	4.3	180 — 220

From the above Table, it is obvious that the cell weight increases with the increase of biotin content, while the amount of 1 - glutamic acid produced is not proportional to the cell weight. There is an optimum point between the cell propagation and the production of 1 - glutamic acid. Therefore, by the appropriate adjustment of the biotin level around 0.5—5 γ/l, 1 - glutamic acid production is effected with high yields.

Various biotin - requesting strains were selected from the bacteria, yeast, and fungi, given in the previously - mentioned Specification No. 9182/57 (Serial No. 849,279) and it has been found that there is a substantially equivalent tendency as in Example 1, when such strains are used.

The new strain which is referred to throughout the specification and claims as "Micrococcus No. 541" has been designated as Micrococcus glutamicus No. 541 and has been deposited in Washington, D.C., U.S.A., with the American Type Culture Collection on the 4th February, 1958, and now bears A.T.C.C. No. 13058.

WHAT WE CLAIM IS:—

1. A method of producing 1 - glutamic acid comprising culturing in a culture medium, which contains saccharidic materials, nitrogen sources, inorganic materials and any other suitable materials, if desired, a microorganism which has biotin - requesting nature, is capable of producing α - ketoglutaric acid from saccharidic materials, and possesses a high activity of 1 - glutamic acid dehydrogenase, particularly a strong activity for reductive amina-

relations with sugar metabolism particularly with fixation and liberation reactions of carbon dioxide.)

Accordingly, to use a biotin - requesting strain and to control the biotin content in a culture medium provides a highly advantageous fermentation control process in such a biochemically complicated fermentation as that producing l - glutamic acid.

According to the invention, therefore, there is provided a method of producing l - glutamic acid, comprising culturing in a culture medium a microorganism which has biotin-requesting nature, is capable of producing α - ketoglutaric acid from saccharidic materials, and possesses a high activity of l - glutamic acid dehydrogenase, particularly a strong activity for reductive amination of the α - ketoglutaric acid, there being present an amount of biotin in the culture medium so as to maintain a favourable balance between the propagation of the microorganism and ferementation, while maintaining the pH value of the culture medium at 6—9 by addition of an acid - neutralising agent, so that l - glutamic acid is produced in the medium in a high yield.

It is well known that biotin is contained in various natural substances, such as egg yolk, kidney, liver, yeast, milk and molasses, and such natural substances and/or their extracts, hydrolysates, and/or digests may be used in place of pure biotin with substantially equivalent results. Consequently, our invention is never limited to the use of pure biotin.

The following Example is given to illustrate the invention.

EXAMPLE 1.

Composition of culture medium by weight.

Glucose	10%
K_2HPO_4	0.1%
$MgSO_4.7H_2O$	0.025%
$FeSO_4.7H_2O$	0.001%
$MnSO_4.4H_2O$	0.001%
Water	ad 100%

Biotin was added to this culture medium in various concentrations. A biotin requiring strain of Micrococcus isolated from soil and named Micrococcus No. 541 was employed for the tests. Micrococcus No. 541 differs from Micrococcus No. 534 given in the previously-mentioned Specification No. 8505/57 in its biotin request only, but has substantially the same morphological, physiological, and other properties as the latter. l - Glutamic acid production with various biotin levels were determined by shaking culture. Table I gives the results after three day cultivation at 28° C., the pH value of the culture medium being maintained in the range from 6.5 to 8.5 by intermittent additions of urea.

TABLE I

Biotin Content (γ/l)	l-glutamic acid (mg/ml)	Dried Cell weight (mg/10 ml)
0.5 — 1.0	20.5	10 — 25
1.0 — 2.5	37.5	25 — 60
2.5 — 5.0	17.0	60 — 120
5.0 — 7.5	6.7	120 — 180
7.5 — 10.0	4.3	180 — 220

From the above Table, it is obvious that the cell weight increases with the increase of biotin content, while the amount of l - glutamic acid produced is not proportional to the cell weight. There is an optimum point between the cell propagation and the production of l - glutamic acid. Therefore, by the appropriate adjustment of the biotin level around 0.5—5 γ/l, l - glutamic acid production is effected with high yields.

Various biotin - requesting strains were selected from the bacteria, yeast, and fungi, given in the previously - mentioned Specification No. 9182/57 (Serial No. 849,279) and it has been found that there is a substantially equivalent tendency as in Example 1, when such strains are used.

The new strain which is referred to throughout the specification and claims as "Micrococcus No. 541" has been designated as Micrococcus glutamicus No. 541 and has been deposited in Washington, D.C., U.S.A., with the American Type Culture Collection on the 4th February, 1958, and now bears A.T.C.C. No. 13058.

WHAT WE CLAIM IS:—

1. A method of producing l - glutamic acid comprising culturing in a culture medium, which contains saccharidic materials, nitrogen sources, inorganic materials and any other suitable materials, if desired, a microorganism which has biotin - requesting nature, is capable of producing α - ketoglutaric acid from saccharidic materials, and possesses a high activity of l - glutamic acid dehydrogenase, particularly a strong activity for reductive amina-

* C L M S *

tion of the α - ketoglutaric acid, there being present an amount of biotin in the culture medium so as to maintain a favourable balance between the propagation of the microorganism and fermentation, while maintaining the pH value of the culture medium at 6—9 by addition of an acid - neutralising agent, so that 1 - glutamic acid is produced in the medium in a high yield.

2. A method according to Claim 1, in which the biotin is supplied by adding a biotin - containing natural substance, its extract, its hydrolysate and/or a digested material obtained therefrom.

3. A method of producing 1 - glutamic acid according to claim 1 or 2, substantially as hereinbefore described.

4. A method of producing 1 - glutamic acid substantially as hereinbefore described in the Example.

5. 1 - Glutamic acid produced according to the method claimed in any of the preceding claims.

CARPMAELS & RANSFORD,
Agents for Applicants,
24, Southampton Buildings, Chancery Lane,
London, W.C.2.

P.S.

Leamington Spa: Printed for Her Majesty's Stationery Office, by the Courier Press, 1960.
Published by The Patent Office, 25, Southampton Buildings, London, W.C.2, from which copies may be obtained.

