

PATENT SPECIFICATION

NO DRAWINGS.

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COMPLETE SPECIFICATION

Process for Producing L-Glutamic Acid by Fermentation

We, SHINSHIN SHOKURYO KOGYO KABUSHIKI KAISHA, a Corporation organized and existing under the Laws of Japan, of 6, 1-chome, Sudacho, Kanda, 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 process for producing L-glutamic acid by fermentation.

Hitherto, L-glutamic acid has been produced mainly by separating it from hydrolyzed wheat protein, soya-bean cake or beet molasses. This method, however, has disadvantages in that not only a fairly expensive source of protein is required but also the apparatus employed in the hydrolysis operation is liable to chemical attack due to the severe conditions under which the hydrolysis is carried out. Although various amino-acids, other than L-glutamic acid, produced as by-products in the process are utilized as as extender for soya-bean cake or as a seasoning, there appears to be a limit to the demand for such by-products.

Krebs and others have advocated the T.C.A. (tricarboxylic acid) cycle as one of the metabolism courses *in vivo*, and it has been shown by experiment that the synthesis of glutamic acid *in vivo* is carried out in accordance with this cycle, and even in a micro-organism, L-glutamic acid is produced in accordance with this cycle.

The present inventors have widely searched in nature for a micro-organism that not only produces L-glutamic acid in the course of its metabolism but also provides an accumulation of said acid external to its metabolic reaction system. The inventors have found that two strains of the genus of *Bacillus* produce and accumulate L-glutamic acid under certain suitable conditions. These two strains of the genus *Bacillus* are known

as *Bacillus circulans* and *Bacillus megatherium*, and can be isolated from the soil. These *Bacilli* may be bred under aerobic conditions on a nutrient culturing medium containing a source of sugar, a source of nitrogen and mineral salts.

Accordingly the present invention provides a process for producing L-glutamic acid, wherein fermentation by the micro-organism *Bacillus circulans* or the micro-organism *Bacillus megatherium* of a culturing medium comprising a source of sugar, a source of nitrogen, and mineral salts, is carried out under aerobic conditions at a temperature of from 27—33° C., a pH value of from 5.0—7.5 and a Kd value of from 1.0— 5.0×10^{-6} , and L-glutamic acid is thereafter separated from the culturing medium. The "Kd value" is the oxygen absorption coefficient. It is defined as the rate at which oxygen is absorbed into a culturing medium and is measured in gram molecules of oxygen absorbed per minute per m.l. of medium.

L-glutamic acid is produced in good yield when the fermentation is performed under aerobic and submerged conditions in a culturing medium containing glucose or an acid-hydrolyzed solution of starch, inorganic ammonium salts or urea, organic nitrogenous substances other than urea and mineral salts. The yield is remarkably increased when a little amount of bran solubles is added to the culturing medium, and ammonium chloride, especially, of all the inorganic ammonium salts, brings about the best result. It has been shown that the density of the culturing medium affects the fermentation and exerts a remarkable influence on the yield.

Thus, by this invention, L-glutamates, which are utilized as seasoning or seasoning constituents, may be produced economically from easily obtainable raw materials. The process of the invention is superior to the

[Price 3s. 6d.]



glutamates/



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former processes in the respect that the procedure is simplified and the raw material can be easily obtained. This process, furthermore, has the advantages over the synthetic processes hitherto adopted in that there is no necessity to resolve a racemic mixture of the glutamic acid because only the *l*-glutamic acid is produced by this process, and there is no chemical reaction under severe conditions which would be liable to damage the apparatus used.

The process of the invention is carried out, for example, in such a manner that either of the two strains of *Bacillus circulans* and *Bacillus megatherium* isolated from the soil is inoculated into a culturing medium containing 5–10% of glucose, 1–2% of ammonium chloride or ammonium nitrate, small amounts of one or more organic nitrogenous substances, and small amounts of mineral salts such as potassium salts, phosphates, magnesium salts and calcium salts. In a preferable embodiment of the invention the organic nitrogenous substances are casein hydrolyzate, bran solubles, peptone and yeast extract, and the mineral salts are potassium dihydrogen phosphate, dipotassium hydrogen phosphate, magnesium sulphate and calcium carbonate. The culturing medium is grown under shaking or aeration-agitation at a temperature of $30^{\circ}\text{C} \pm 3^{\circ}\text{C}$. and a pH value in the range of 5.5–7.5. All the percentages mentioned above and hereinafter are by weight. The fermentation is stopped when

the accumulated volume of *l*-glutamic acid seems to have reached the maximum, and the solution after the completion of the fermentation is filtered centrifugally. The resultant filtrate is then concentrated under reduced pressure, adjusted to a pH value of 3.1–3.3 and allowed to stand at a lower temperature. The *l*-glutamic acid thereby extracted from the filtrate is separated centrifugally and is purified by washing with water.

The following examples illustrate the process of this invention.

EXAMPLE 1

A culturing solution with a pH value of 6.8 and containing 5% of glucose; 1.0% of ammonium chloride; 0.12% of potassium dihydrogen phosphate, 0.06% of magnesium sulphate and 1% of calcium carbonate, as mineral salts; and 0.02% of casein enzymatic-hydrolyzate and 0.02% of bran solubles as organic nitrogenous substances; was introduced in 100 ml. quantities into a 500 ml. flat-shouldered kolben flask. An inoculum of *Bacillus megatherium*, prepared 24 hours before in a similarly composed culturing solution at a temperature of from 27 to 33°C ., was added in 5 ml. quantities into said kolben flask and was cultured on a reciprocating type of shaker at a temperature of 30°C ., at a Kd value of from 3.0 to 5.0×10^{-6} .

The fermentation process proceeded as follows:—

Time in hours	Sugar remaining %	<i>l</i> -glutamic acid produced %	pH
24	2.30	0.35	6.7
48	1.60	0.78	5.5
72	0.85	0.91	5.5
96	0.06	0.88	5.8

After 96 hours the culturing solution was filtered centrifugally to remove the cells of the *Bacillus* and other suspended matters. The resultant filtrate was concentrated under a reduced pressure to one tenth of its original volume, adjusted to a pH value of 3.2, kept in an ice-room for 24 hours and was then filtered. 8.5 gms. of crude crystallized *l*-glutamic acid were obtained from each litre of the mother liquor.

EXAMPLE 2

An inoculum was prepared by growing *Bacillus circulans*, isolated from soil, in a culturing solution having a pH value of 6.8 and a temperature of from 27 to 33°C . and containing 2% of glucose; 0.2% of peptone and 0.05% of casein hydrolyzate as organic nitrogenous compounds; 0.5% of NH_4Cl ; 0.1%

of KH_2PO_4 and 0.06% of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ as mineral salts, and there was added, after being sterilized, 0.5% of sterilized CaCO_3 . 250 ml. of this inoculum was added to 5 litres of a culturing medium, of which the composition was as described below, and the inoculum was bred under submerged culture while being given an aeration-agitation. The condition of the aeration-agitation was Kd = 3.0×10^{-6} and the temperature was 30°C .

The composition of the culturing medium was as follows:—

glucose 5%, ammonium chloride 1.2%, casein hydrolyzate 0.05%, K_2HPO_4 0.12%, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.06%, bran solubles 0.012%, yeast extract 0.05%, and CaCO_3 1%. The pH of the culturing medium was 6.8.

The fermentation process proceeded as follows:—

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40

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60

65

70

95

100

105

110

	Time in hours	Sugar remaining %	<i>l</i> -glutamic acid produced %	pH
	24	2.75	0.56	6.7
	48	2.00	0.81	5.2
5	72	1.08	1.09	5.4
	96	0.62	1.49	6.0

After 96 hours from the beginning of the culturing process, the culturing solution was boiled for five minutes and was filtered centrifugally to remove the cells of the *Bacillus* and other suspended matters. The resultant filtrate was concentrated under reduced pressure to one fifth of its original volume, adjusted to a pH value of 3.2, kept in an ice-room for 24 hours and was then filtered. In consequence, 13 gms. of crystallized *l*-glutamic acid were obtained from each litre of the mother liquor.

WHAT WE CLAIM IS:—

1. A process for producing *l*-glutamic acid, wherein fermentation by the micro-organism *Bacillus circulans* or the micro-organism *Bacillus megatherium* of a culturing medium comprising a source of sugar, a source of nitrogen, and mineral salts is carried out under aerobic conditions at a temperature of from 27—33° C., a pH value of from 5.0—7.5 and a K_d value of from 1.0—5.0×10⁻⁶, and *l*-glutamic acid is thereafter separated from the culturing medium.

2. A process according to Claim 1, wherein the source of nitrogen is an ammonium salt, and/or one or more organic nitrogenous substances.

3. A process according to Claim 1 or 2, wherein the culturing medium has a pH value of from 5.5—7.5 and comprises 5—10% of glucose, 1—2% of ammonium chloride or ammonium nitrate, and small amounts of potassium salts, phosphates and magnesium salts, and small amounts of organic nitrogenous substances.

4. A process according to any of the preceding claims, wherein the culturing medium contains small amounts of the mineral salts potassium dihydrogen phosphate, dipotassium hydrogen phosphate, magnesium sulphate and calcium carbonate, and small amounts of

one or more of the organic nitrogenous substances casein enzymatic-hydrolyzate, bran solubles, peptone and yeast extract.

5. A process according to any of the preceding claims, wherein the *Bacillus* employed therein is bred by inoculating said *Bacillus*, isolated from the soil, at a temperature of from 27 to 33° C., into a culturing medium having a pH of from 5.0—7.5 and comprising a source of sugar, a source of nitrogen and mineral salts.

6. A process according to Claim 5, wherein the *Bacillus megatherium* is bred by inoculating said *Bacillus* into a culturing medium having a pH value of 6.8 and a temperature of from 27—33° C. and comprising 5% of glucose, 1% of ammonium chloride, 0.12% of potassium dihydrogen phosphate, 0.06% of magnesium sulphate, 0.02% of casein enzymatic-hydrolyzate, 0.02% of bran solubles and 1% of calcium carbonate.

7. A process according to Claim 5, wherein the *Bacillus circulans* is bred by inoculating said *Bacillus* into a culturing medium having a pH value of 6.8 and a temperature of from 27 to 33° C. and comprising 2% of glucose, 0.2% of peptone, 0.05% of casein hydrolyzate, 0.5% of ammonium chloride, 0.1% of potassium dihydrogen phosphate, 0.06% of magnesium sulphate .7 H₂O and 0.5% of calcium carbonate.

8. A process for producing *l*-glutamic acid, substantially as hereinbefore described with reference to Example 1 or 2 of the foregoing examples.

9. *l*-glutamic acid whenever produced by a process claimed in any of the preceding claims.

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	Time in hours	Sugar remaining %	<i>l</i> -glutamic acid produced %	pH
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After 96 hours from the beginning of the culturing process, the culturing solution was boiled for five minutes and was filtered centrifugally to remove the cells of the *Bacillus* and other suspended matters. The resultant filtrate was concentrated under reduced pressure to one fifth of its original volume, adjusted to a pH value of 3.2, kept in an ice-room for 24 hours and was then filtered. In consequence, 13 gms. of crystallized *l*-glutamic acid were obtained from each litre of the mother liquor.

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1. A process for producing *l*-glutamic acid, wherein fermentation by the micro-organism *Bacillus circulans* or the micro-organism *Bacillus megatherium* of a culturing medium comprising a source of sugar, a source of nitrogen, and mineral salts is carried out under aerobic conditions at a temperature of from 27—33° C., a pH value of from 5.0—7.5 and a Kd value of from $1.0-5.0 \times 10^{-2}$, and *l*-glutamic acid is thereafter separated from the culturing medium.
2. A process according to Claim 1, wherein the source of nitrogen is an ammonium salt, and/or one or more organic nitrogenous substances.
3. A process according to Claim 1 or 2, wherein the culturing medium has a pH value of from 5.5—7.5 and comprises 5—10% of glucose, 1—2% of ammonium chloride or ammonium nitrate, and small amounts of potassium salts, phosphates and magnesium salts, and small amounts of organic nitrogenous substances.
4. A process according to any of the preceding claims, wherein the culturing medium contains small amounts of the mineral salts potassium dihydrogen phosphate, dipotassium hydrogen phosphate, magnesium sulphate and calcium carbonate, and small amounts of

one or more of the organic nitrogenous substances casein enzymatic-hydrolyzate, bran solubles, peptone and yeast extract.

5. A process according to any of the preceding claims, wherein the *Bacillus* employed therein is bred by inoculating said *Bacillus*, isolated from the soil, at a temperature of from 27 to 33° C., into a culturing medium having a pH of from 5.0—7.5 and comprising a source of sugar, a source of nitrogen and mineral salts.

6. A process according to Claim 5, wherein the *Bacillus megatherium* is bred by inoculating said *Bacillus* into a culturing medium having a pH value of 6.8 and a temperature of from 27—33° C. and comprising 5% of glucose, 1% of ammonium chloride, 0.12% of potassium dihydrogen phosphate, 0.06% of magnesium sulphate, 0.02% of casein enzymatic-hydrolyzate, 0.02% of bran solubles and 1% of calcium carbonate.

7. A process according to Claim 5, wherein the *Bacillus circulans* is bred by inoculating said *Bacillus* into a culturing medium having a pH value of 6.8 and a temperature of from 27 to 33° C. and comprising 2% of glucose, 0.2% of peptone, 0.05% of casein hydrolyzate, 0.5% of ammonium chloride, 0.1% of potassium dihydrogen phosphate, 0.06% of magnesium sulphate .7 H₂O and 0.5% of calcium carbonate.

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