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PATENT SPECIFICATION

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COMPLETE SPECIFICATION

Fermentation process for preparing L-Glutamic Acid

We, AJINOMOTO CO. INC., of 7, 1-chome, Takara-cho, Chuo-ku, Tokyo, Japan, a corporation organised under the laws of Japan, and SANRAKU DISTILLERS CO. INC., of 7, 1-chome, Takara-cho, Chuo-ku, Tokyo, Japan, a corporation organised under the laws of 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 the production of L-glutamic acid by fermentation.

Some species of *geni Bacillus*, *Micrococcus*, *Corynebacterium* and *Microbacterium* can produce L-glutamic acid in a medium comprising a carbon source, (for example glucose, fructose, maltose, sucrose or starch which has been hydrolysed by acids or enzymes), a nitrogen source, (for example ammonium hydroxide, ammonia, ammonium sulphate, ammonium chloride, ammonium nitrate or urea) inorganic materials (for example KH_2PO_4 , MgSO_4 , FeSO_4 or MnSO_4) and nutrients (for example corn steep liquor, hydrolysed protein solution, amino acids, biotin, desthio-biotin, diamino-perlarginic acid or oleic acid) at a temperature of from 27 to 32° C.

Preferred methods of producing L-glutamic acid are described in, e.g., Specifications Nos.

873,448, 876,943, 889,187, 905,973, 920,241 and 920,242.

It is an object of this invention to provide a process for producing L-glutamic acid in a high yield and in a short time.

The present invention provides a process for the production of L-glutamic acid, which comprises culturing microorganisms capable of producing L-glutamic acid in a nutrient medium containing an assimilable carbon source and an assimilable nitrogen source, while shaking under aerobic conditions, at a temperature at which multiplication of the microorganisms takes place in the fermentation, subsequently feeding a 44—49 g/dl sugar solution to the culture medium and recovering L-glutamic acid from the culture medium.

Thus, the fermentation of L-glutamic acid comprises two stages:

- (1) an initial multiplicative stage when the number of bacteria is increased, and
- (2) the L-glutamic acid production stage.

We have succeeded in producing L-glutamic acid rapidly and efficiently by this process. Preferably, the medium is heated to 34—38° C before, during or after feeding the 44 to 49 weight/vol % sugar solution to the culture medium.

Table 1 shows the amount of L-glutamic acid produced, the yield of L-glutamic acid, calculated on the total sugar, and the time at which the sugar was fed.

TABLE 1

Feeding time on medium initially containing 15g/dl of sugar	L-glutamic acid produced (g/dl)	Yield of L-glutamic acid (% by weight)
after 3g/dl (i.e. 20%) of the initial sugar decrease had been used	5.50	29.0
after 6 g/dl of the initial sugar had been used	7.03	37.0
after 9 g/dl of the initial sugar had been used	6.10	32.1

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The medium used in the above and subsequent Tables contained 0.1 g/dl of KH_2PO_4 , 0.04 g/dl of

- 5 $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 2 p.p.m of each of Fe^{++} and Mn^{++} , 0.5 g/dl of corn steep liquor, 80 γ /l of vitamin B_{12} , 15 g/dl of initial sugar and 19 g/dl of sugar after feeding.
- 10 *Brevibacterium lactofermentus* No. 2256 (ATCC No. 13869) was inoculated on the medium and cultured for 48 hours at 30° C while shaking. The A.T.C.C. number refers to the accession number in the American Type Culture Collection.

From the above Table, it is seen that the best time for feeding the high concentration sugar solution is after a 30 to 54 percent decrease in the initial sugar content. In this Specification, the term "sugars" means glucose, fructose, maltose, sucrose and starch hydrolysate.

Table II shows the amount of L-glutamic acid produced, the yield of L-glutamic acid calculated on total sugar content, and the culturing time corresponding to a certain temperature of the culture medium fed with high concentration sugar solution.

TABLE II

Temperature (°C.)	L-glutamic acid produced (g/dl)	Yield based on total sugar (% by weight)	Culturing duration (hours)
30	7.45	36.5	50
34	8.01	39.3	44
36	8.87	43.5	40
39	6.57	32.2	44

- 30 From the above Table, it is seen that the optimum temperature for the culture medium at the L-glutamic acid production stage is 34—38° C.

Table III shows the total amount of sugar,

the amount of L-glutamic acid produced, the yield calculated on the total amount of sugar, the culture time corresponding to a certain sugar feeding and the temperature of culture medium at a later stage in the fermentation.

TABLE III

Sugar Feeding	Temperature (°C.)	Total L-glutamic sugar acid produced		Yield based on total sugar (% by weight)	Culturing duration (hours)
		(g/dl)	(g/dl)		
no feed	31.5	14.70	5.89	40.0	40
no feed	36	14.70	6.21	42.2	36
no feed	36	20.16	7.52	37.3	42
feed	36	20.16	8.55	42.4	36

- 40 From the above table, it is seen that the L-glutamic acid fermentation may be carried out rapidly and efficiently only when the high concentration sugar solution is fed and the temperature of the medium is raised, both at a late stage in the fermentation.

- 45 Table IV shows the total amount of sugar

supplied, the amount of L-glutamic acid produced, the yield calculated on the total amount of sugar and the culturing time corresponding to a feed sugar quantity. In this case the temperature was raised to 36° C. after feeding the sugar.

TABLE IV

Amount of sugar to initial sugar	Total sugar supplied (g/dl)	L-glutamic acid produced (g/dl)	Yield based on total sugar (%)	Culturing duration (hours)
1/4	20.79	8.40	41.5	36
2/5	26.28	6.78	25.8	50
1/5 in twice	24.70	7.32	29.6	53
1	32.03	5.35	16.7	50

From the above Table, it is seen that the total amount of sugar supplied to the culture medium should preferably be 21—23 g/dl and less. If the total amount of sugar supplied to the culture medium is 30 g/dl, the sugar metabolism decreases considerably.

Any microorganisms capable of producing L-glutamic acid from carbon compounds and nitrogen compounds can be used in the process of the present invention. The following are preferred for the industrial production of L-glutamic acid: species belonging to the genus *Bacillus* such as *Bacillus megatherium*, *Bacillus subtilis*, *Bacillus circularis*, *Bacillus cereus*; species *Escherichia coli*; species belonging to the genus *Micrococcus* such as *Micrococcus varians* and species belonging to the genus *Brevibacterium* such as *Brevibacterium lactofermentus*, (for example ATCC Nos. 13655 and 13869), *Brevibacterium kawasakii*, *Brevibacterium devarticatum*, *Brevibacterium aminogenes* and *Brevibacterium saccharolyticum* (for example ATCC No. 14066).

The process for producing L-glutamic acid according to the present invention has been compared with the generally adopted processes and shows the following advantages: (1) a high concentration sugar solution can be used, and consequently L-glutamic acid is accumulated in a high concentration; (2) the culturing time is decreased; and (3) despite the high concentration of the sugar solution, the yield of L-glutamic acid based on total amount of sugar is high.

The following Examples illustrate the invention:

EXAMPLE 1.

Starch hydrolysate solution which had been sterilised at 115° C. for 15 minutes was made up to 15 kilolitres. The medium contained sugar corresponding to 15 g/dl of glucose, 0.1 g/dl of KH_2PO_4 , 0.04 g/dl of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ p.p.m. of Fe ions and 2 p.p.m. of Mn ions, 5 γ /l of biotin, 0.5 g/dl of corn steep liquor and 50 γ /l of vitamin B.

Brevibacterium lactofermentus strain no. 2256 (A.T.C.C. No. 13869) was inoculated on

the medium, which had been adjusted with ammonia gas to pH 7.8, and was cultured at 31° C. while shaking under aerobic conditions. The pH of the medium was maintained in the alkaline range by introducing gaseous ammonia with air during the incubation.

At a later stage in the multiplication of the bacteria when 7.5 g/dl of the initial sugar had been used (i.e., after 16 hours), the medium was heated to 36° C., and after 17 hours 1.1 kilolitre of 46.6% sugar solution was fed to the culture medium. The medium was then cultured at 36° C. for 44 hours. The total amount of sugar used as 19.5 g/dl.

The amount of L-glutamic acid produced was 8.49 g/dl and the yield calculated on total sugar content was 43.5 percent. The supernatant liquid from which the microorganisms were then centrifuged, was concentrated, adjusted to pH 3.2 and subjected to crystallisation. The L-glutamic acid obtained weighed 507 kilograms.

EXAMPLE 2.

Brevibacterium saccharolyticum strain no. 7636 was inoculated on the medium which had been prepared in the same way as Example 1, and was cultured under the same conditions as in Example 1. When 8 g/dl of the initial sugar in the medium had been used, 0.6 kilolitres of 44.17% sugar solution was fed to the culture medium, which was then heated to 36° C. When a further decrease of 3% of the initial sugar in the medium had occurred, after 22 hours from the beginning of the fermentation, 0.6 kilolitres of the same sugar solution was fed once again (sugar concentration: 19.71 g/dl calculated on total sugar used).

The amount of L-glutamic acid produced was 8.27 g/dl. The yield calculated on total amount of sugar was 42.0 percent. The L-glutamic acid was crystallised and weighed 572 kilograms.

EXAMPLE 3.

Brevibacterium flavum strain no. 3423 was cultured in the same way as in Example 1, and after the initial sugar content had de-

creased by 6.5 g/dl, (i.e., after 16 hours from the beginning of the fermentation), 1 kilolitre of 49.00% sugar solution was fed to the culture medium (the total amount of sugar used is 19.27 g/dl). Subsequently, the medium was heated to 38° C. after 17 hours. The fermentation took 48 hours.

The amount of L-glutamic acid produced was 8.71 g/dl and the yield calculated on the total amount of sugar was 45.2 per cent. The L-glutamic acid was crystallised and weighed 574 kilograms.

EXAMPLE 4.

Twenty millilitres of a culture medium was prepared and sterilised in a 500 millilitres flask; the contained sugar corresponding to 10 g/dl of glucose, 6 γ /l of biotin, 0.1 g/dl of KH_2PO_4 , 0.04 g/dl of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.5 ml/dl of soya bean protein hydrolysate, 0.6 g/dl of urea, 2 p.p.m. of Fe ions and 2 p.p.m. of Mn ions.

Brevibacterium lactofermentus strain no. 2256 was inoculated on the medium and cultured in the same way as Example 1. On culturing, the medium was maintained in alkaline range by adding 0.5 ml of 45 per cent urea solution. After 20 hours, 5.5 ml of 45.0 per cent molasses solution was fed to the medium and was cultured at 34° C. The fermentation took 42 hours.

The amount of L-glutamic acid produced was 7.3 g/dl and the yield was 40.1 per cent calculated on the total amount of sugar.

WHAT WE CLAIM IS:—

1. A process for the production of L-glutamic acid, which comprises culturing microorganisms capable of producing L-glutamic acid in a nutrient medium containing an assimilable carbon source and an assimilable nitrogen source, while shaking under aerobic conditions, at a temperature at which multiplication of the microorganisms takes place in the fermentation, subsequently feeding a 44—49 g/dl sugar solution to the culture medium and recovering L-glutamic acid from the culture medium.

2. A process as claimed in claim 1, wherein, before, during or after feeding the sugar solution, the medium is heated to 34 to 38° C.

3. A process as claimed in claim 1 or claim 2, wherein the high concentration sugar solution is added after the carbon source has decreased by 30 to 54 percent by weight.

4. A process as claimed in any of claims 1 to 3, wherein the microorganism belongs to genus *Brevibacterium*, genus *Bacillus*, genus *Micrococcus* or species *Escherichia coli*.

5. A process for the production of L-glutamic acid, substantially as described with reference to any of the Examples.

6. L-glutamic acid when produced by the process claimed in any of the preceding claims.

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4. A process as claimed in any of claims 1 to 3, wherein the microorganism belongs to genus *Brevibacterium*, genus *Bacillus*, genus *Micrococcus* or species *Escherichia coli*.

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