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COMPLETE SPECIFICATION

NO DRAWINGS



Chuo-ku

A Process for Producing L-Glutamic Acid

We, AJINOMOTO CO. INC. of 7, 1-chome, Takara-cho, ~~Chiyoda-ku~~ Tokyo, Japan, a corporation organised under the laws of Japan and SANRAKU OCEAN 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.

Many microorganisms are known to be capable of producing glutamic acid by fermentation from suitable carbon and nitrogen sources. Glucose is the carbon source in the usual industrially employed fermentation processes. The nitrogen source may be ammonia, ammonium sulphate, ammonium nitrate or urea or a mixture thereof. The glutamic acid is produced when any of a wide variety of microorganisms is cultured aerobically at a temperature between 27°C and 33°C on a nutritive medium containing such carbon and nitrogen sources together with certain well known minor constituents beneficial for the metabolism of the microorganisms.

Glucose is a relatively costly source of carbon and it is an object of this invention to provide a process for the production of L-glutamic acid using a less costly carbon source for media on which the glutamic acid producing microorganisms can be cultured.

Cane molasses, sugar beet molasses, raw cane or beet sugar, cane juice (thin juice) and extracted beet juice (raw juice), are available abundantly at low cost. The commercial molasses and the sugars contain about 50% weight/volume of sucrose. Attempts have been made to employ the sucrose of mol-

asses and other sugars as a carbon source for the fermentative production of glutamic acid. These attempts have not resulted in a practical industrial process for the two following primary reasons: (i) Molasses and other sugars contain coloured substances which inhibit the fermentative production of glutamic acid by all normally employed microorganisms; (ii) Molasses and other sugars further contain substantial amounts of biotin and of other substances which promote the growth of the microorganisms at the expense of their glutamic acid producing power. Therefore, in spite of its low cost, molasses and other sugars have not heretofore been an economical carbon source for glutamic acid fermentation.

We have now found that the metabolism of microorganisms capable of producing glutamic acid can be limited by adding surface agents to the culture media comprising molasses or other sugars as the carbon source. Glutamic acid can be produced in high yields and high concentration in culture media which contain molasses or other sugars and surface active agents.

The present invention therefore provides a process for producing L-glutamic acid, which comprises cultivating a microorganism capable of producing L-glutamic acid in the presence of beet molasses, cane molasses, thin cane juice, raw cane sugar, raw beet sugar or raw beet juice and in the presence of up to 1% wt./vol. calculated on the volume of the medium, of a surface active agent.

Throughout this specification the relationship between % by wt. and % by vol. is the same as that existing between grams and decilitres.

The term "a microorganism capable of producing L-glutamic acid", as used herein, means a microorganism which is in use in

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the production of L-glutamic acid by fermentation, or whose use in such processes is described in the literature. Examples of literature which describes the use of microorganisms capable of producing L-glutamic acid are U.K. Specifications Nos. 873,448; 876,943; 889,187; 905,973; 920,241 and 920,242 and some of the microorganisms referred to herein, in the Examples, are disclosed in the above-mentioned U.K. Specifications.

A preferred amount of surface active agent is 0.01 to 0.1% by weight of the medium.

The following Table 1 lists the results of experiments in which several surface active agents were added to culture media which contain 10 g/dl of beet molasses (sucrose), 0.1 g/dl of KH_2PO_4 , 0.04 g/dl of MgSO_4 .

7H₂O and 0.6 g/dl of urea. The media were inoculated with *Brevibacterium lactofermentum* strain No. 2256 (ATCC No. 13869), and cultured at 30°C for 48 hours with shaking. The A.T.C.C. and N.R.R.L. numbers used herein refer to the accession numbers of the microorganisms in the American Type Culture Collection and in Northern Regional Research Laboratories of the U.S. Department of Agriculture, respectively.

The pH of the media was maintained in the alkaline range by adding a 45% wt./vol. urea solution. The first column of the Table gives the trade name of the surface active agent used. The second column shows the initial concentration of the surface active agent in percent by weight. The third column shows the concentration of glutamic acid produced in the media.

Table 1

Surface Active Agent	Surface Active Agent Concentration (% weight volume)	Glutamic Acid Concentration g/dl
(None)	0	1.28
Tween 20	0.01	2.81
" "	0.05	2.25
" "	0.1	2.41
" 40	0.01	2.22
" "	0.05	2.68
" "	0.1	2.91
" 80	0.001	2.17
" "	0.01	2.13
" "	0.1	2.18
Span 20	0.01	1.83
" "	0.05	2.71
" "	0.1	2.99
Aldo 28	0.01	2.72
" "	0.1	3.57
" "	1.0	2.17

Note:

- Tween 20 is a polyoxyethylene sorbitan monolaurate
 Tween 40 is a polyoxyethylene sorbitan monopalmitate
 Tween 80 is a polyoxyethylene sorbitan monooleate
 Span 20 is a sorbitan monolaurate
 Aldo 28 is a glycerol monostearate
 "Tween" and "Span" are Registered Trade Marks.

The effect of the surface active agent on the growth of glutamic acid producing microorganisms is illustrated in Table 2 which lists results of tests performed on microorganisms grown on media comprising molasses and certain amounts of a surface active agent. In all tests, a culture medium contains 10 g/dl of molasses, 0.1 g/dl of KH_2PO_4 , 0.04 g/dl of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 2 p.p.m. of

Fe ion and 2 p.p.m. of Mn ion, 0.8 g/dl of urea, 5 / 1 of biotin and 0.5 ml/dl of soya bean protein hydrolysate. The microorganism employed was *Brevibacterium lactofermentum* Strain No. 2256 (ATCC. No. 13869). After 42 hours incubation, the bacterial growth was determined by measuring the turbidity of the culture at a wavelength of 562 millimicrons.

Table 2

Surface Active Agent and Concentration in % wt/vol.		Turbidity of Culture	Residual sucrose Concentration g/dl
(None)	0	0.59	0.71
5 Span 20	0.01	0.61	0.71
" "	0.1	0.51	5.05
" 60	0.05	0.57	1.38
Tween 60	0.15	0.62	2.48
Tween 60	0.01	0.61	0.67
10 Aldo 28	0.05	0.48	5.03
	0.01	0.65	0.71
	0.2	0.62	3.21

Note:

Span 60: is a sorbitan monostearate

15 As is seen from Table 2, the surface active agent does not inhibit the growth of bacteria producing glutamic acid.

When too much surface active agent is added to a medium, the microorganisms can not metabolise to produce glutamic acid. However, it is evident from Table 3 that addition of surface active agent unfavourably affects the proliferation of microorganisms.

25 Therefore, the metabolism of microorganism producing glutamic acid can be controlled favourably to produce glutamic acid in high yield with inactivating the glutamic acid producing enzyme in the cell.

30 Accordingly, on addition of an optimum amount of surface active agent to culture media, glutamic acid fermentation occurs by metabolising sucrose, and the consumed sucrose can be efficiently converted into glutamic acid.

35 Table 3 shows that an excessive or an insufficient initial concentration of surface active agent reduces the yield. The culture medium in each test contained 10 g/dl of beet molasses, 0.1 g/dl of KH_2PO_4 , 0.04 g/dl of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, and 0.6 g/dl of urea. The surface active agent used was Tween 60. The incubation proceeded for 48 hours at 30°C, while shaking.

Table 3

Surface Active Agent Concentration in % (wt/vol.)	Residual Sugar (g/dl)	Glutamic Acid Produced (g/dl)
0.001	0.66	2.25
50 0.005	0.72	2.31
0.01	0.69	2.52
0.05	0.92	3.86
0.1	1.93	4.00
0.3	3.00	2.30
55 0.5	6.11	1.50
1.0	9.95	—

60 Glutamic acid is obtained in a high yield and high concentration when the surface active agent, for example a non-ionic surface active agent, is added to culture media in the highest concentration not so as to inhibit the proliferation of microorganisms.

The reason why L-glutamic acid is not accumulated in a culture medium when molasses, raw sugars or cane or beet juice are used as such, is that these materials contain a large amount of growth promoting factors for L-glutamic acid producing microorganism such as microorganisms belonging to the genera *Micrococcus*, *Brevibacterium*, *Corynebacterium*, *Bacillus* and *Microbacterium*. The following Table shows the contents of growth promoting factors for L-glutamic acid producing microorganisms in several natural raw sugar materials, when converted to equivalent amounts of biotin.

TABLE

raw material	contents (γ/100 grams of sugar)	
raw cane sugar	20 - 40	85
thin cane juice	30 - 50	
cane molasses (final or black-strap)	300 - 700	
raw beet sugar	50 - 70	80
beet molasses	30 - 40	

The following Examples illustrate the invention, the weight/volume percentages being grams/decilital.

Example 1

Beet molasses containing 67 g/dl of sucrose was diluted with water to approximately twice its initial volume, diatomaceous earth was added to the diluted molasses which was then filtered. The filtrate was diluted with water to a sucrose concentration of 10 g/dl and a culture medium was prepared from this filtrate by adding 0.1 g/dl of potassium di-hydrogen phosphate, 0.04 g/dl of magnesium sulphate heptahydrate, 0.6 g/dl of urea and 0.1 g/dl of the surface active

agent Tween 60. The pH of the medium was adjusted to 7.0 with aqueous ammonia solution. 20 ml batches of the culture medium were placed in 500 ml flasks, sterilised at

- 5 120°C for 15 minutes, and inoculated with *Brevibacterium lactofermentum* Strain No. 2256 (ATCC No. 13869), which had previously been cultured for 24 hours. The fermentation was allowed to proceed at 31°C
10 for 48 hours while shaking. A 45% weight/volume aqueous solution of urea was added to each flask during fermentation to keep the culture medium slightly alkaline. The medium after fermentation contained 4.00
15 g/dl of L-glutamic acid. The culture broth obtained after fermentation was filtered in order to separate cells, concentrated under reduced pressure, and its pH was adjusted to the isoelectric point of glutamic acid (3.2)
20 by means of hydrochloric acid. When the adjusted broth was kept overnight in an ice box, 33.5 g of crystallised crude L-glutamic acid were obtained from each litre of the original broth.

25 Example 2

- An aqueous solution of sugar having a sucrose concentration of 10 g/dl, which was prepared in the manner described in Example 1, was treated with 0.1 g/dl of potassium di-
30 hydrogen phosphate, 0.04 g/dl of magnesium sulphate heptahydrate, 0.6 g/dl of urea and 0.1 g/dl of the surface active agent Aldo 28, to form a culture medium. The pH of the medium was adjusted to 7.0 with aqueous
35 ammonia solution. 20 ml batches of the culture medium were placed in 500 ml volume flasks, sterilised, and then inoculated with previously prepared *Brevibacterium flavum* Strain No. 2247 (ATCC No. 14067).
40 The fermentation was allowed to proceed at 31°C for 42 hours while shaking. A 45% weight/volume aqueous solution of urea was added to each flask during the fermentation to keep the culture medium slightly alkaline.
45 From each litre of the broth 28 grams of crystallised crude L-glutamic acid were obtained in the manner described in Example 1.

Example 3

- 50 Ten litres of an aqueous solution of beet molasses were diluted with a same volume of water while stirring thoroughly. 500 grams of diatomaceous earth were added to the diluted solution, which was thereafter suction
55 filtered. A culture medium was prepared from 6.6 litres of the filtrate, 14 grams of potassium dihydrogen phosphate, 5.6 grams of magnesium sulphate heptahydrate and 14 grams of the surface active agent Tween 60.
60 The pH of medium was adjusted to 7.0, diluted to 13.3 litres and placed in a 20 litre stainless steel fermentation jar. The medium in the jar was sterilised at 115°C for 20 minutes, cooled, and inoculated with 700 ml
65 of a seed liquor of *Brevibacterium lactofer-*

mentum, Strain No. 2256 (ATCC No. 13869), which had been cultured for 15 hours. Fermentation was carried out in the jar at 31°C under aeration and agitation. During fermentation, gaseous ammonia together with
70 air was introduced into the jar whereby the pH value of the culture medium was kept at approximately 7.8. 48 hours after inoculation, fermentation was completed and the concentration of L-glutamic acid in the fer-
75 mentation broth was 4.38 grams per decilitre. The broth was filtered after addition of 1.5 percent weight/volume of diatomaceous earth to remove the bacterial cells. The filtrate was decolourised by addition of activated carbon, and finally concentrated under reduced pressure. When the residue was acidified to pH 3.2 with hydrochloric acid and cooled overnight in an ice box, 510
80 grams of crude L-glutamic acid were pre-
85 cipitated and recovered by filtration.

Example 4

A culture medium containing 12 g/dl of sugar beet molasses, 0.1 g/dl of diammonium hydrogen phosphate, 0.04 g/dl of mag-
90 nesium sulphate heptahydrate and 0.6 g/dl of urea, was prepared in the manner described in Example 1. 20 ml batches of the culture medium were placed in 500 ml
95 volume flasks, sterilised and then inoculated with *Brevibacterium lactofermentum* Strain No. 2256 (ATCC No. 13869) which had been prepared by culturing for 24 hours. The fermentation was allowed to proceed at 31°C while shaking. An aqueous solution of
100 urea was added to each flask during fermentation to keep the culture medium slightly alkaline. Six hours after inoculation, the flasks were divided to two groups and one
105 group (first group) was treated with the surface active agent Tween 60 in a concentration 0.05% weight/volume and fermentations of both groups were continued. Another group (second group) was treated with surface active agent Tween 60 after ten hours
110 from inoculation in a concentration 0.1% weight/volume and fermentation was continued. The additions of surface active agent were made under sterilised condition. Sixty
115 three hours after inoculation, fermentation
of both groups was completed. The concentration of L-glutamic acid accumulated in the broth was 5.56 g/dl in the first group and 4.24 g/dl in the second group.

Example 5

120 Crystals of raw beet sugar were dissolved in water to give a 20% solution and diatomaceous earth was added to the said solution which was then filtered. The filtrate was diluted with water to a sucrose concentration
125 of 10 g/dl, then a culture medium was prepared from this solution by the addition of 0.3 g/dl of potassium dihydrogen-phosphate, 0.04 g/dl of magnesium sulphate heptahydrate, 0.6 g/dl of urea and 0.04 g/dl of the
130

surface active agent Tween 60. The pH of the medium was adjusted to 7.0 with aqueous ammonia solution. 20 ml batches of the culture medium were placed in 500 ml flasks, sterilised at 120°C for 15 minutes, and inoculated with *Brevibacterium lactofermentum*, Strain No. 2256 (ATCC No. 13869), which had been cultured for 24 hours. The fermentation was allowed to proceed at 31°C for 48 hours while shaking. A 45% weight/volume aqueous solution of urea was added to each flask during fermentation to keep the culture medium slightly alkaline. The medium after fermentation contains 3.87 g/dl of L-glutamic acid. The culture broth obtained after fermentation was filtered in order to separate cells, concentrated under reduced pressure, and its pH was adjusted to the isoelectric point of glutamic acid (3.2) with hydrochloric acid. When the adjusted broth was kept overnight in an ice box, 34.1 g of crystalline crude L-glutamic acid were obtained from each litre of the original broth.

Example 6

Crystals of raw cane sugar were dissolved in water, and diatomaceous earth was added to said solution which was then filtered. The filtrate was further diluted with water to a sucrose concentrate of 10 g/dl, then a culture medium was prepared from this sugar solution by the addition of 0.2 g/dl of diammonium hydrogen phosphate, 0.04 g/dl of magnesium sulphate heptahydrate, 0.6 g/dl of urea and 0.03 g/dl of the surface active agent Aldo 28. The pH of the medium was adjusted to 7.0 with aqueous ammonia solution. 20 ml batches of the culture medium were placed in 500 ml flasks, sterilised and inoculated with *Brevibacterium flavum*, Strain No. 2247 (ATCC No. 14067), which had been cultured previously. The fermentation was allowed to proceed at 31°C for 42 hours while shaking. An aqueous solution of urea was added to each flask during fermentation to keep the culture medium slightly alkaline. 27 grams of crystalline crude L-glutamic acid were obtained from each litre of the original broth in the manner described in Example 1.

Example 7

An L-glutamic acid fermentation was carried out in the manner described in Example 6 using thin juice of sugar cane (expelled juice by press of sugar cane) and 0.05 g/dl of the surface active agent Span 60 instead of the raw cane sugar and surface active agent Aldo 28 used in Example 6. The concentration of L-glutamic acid in the fermentation broth was 3.54 g/dl.

Example 8

1.4 kilograms of raw cane sugar were dissolved with 10 litres of water and 500 grams of diatomaceous earth were added to said solution, which was thereafter suction filtered. A culture medium was prepared from

6.6 litres of the filtrate, 28 grams of potassium dihydrogen phosphate, 5.6 grams of magnesium sulphate heptahydrate and 4.2 grams of the surface active agent Tween 60. The pH of medium was adjusted to 7.0, diluted to 13.3 litres and placed in a 20 litre stainless steel fermentation jar. The medium in the jar was sterilised at 115°C, for 20 minutes, cooled, and inoculated with 700 ml, of a seed liquor of *Brevibacterium lactofermentum*, Strain No. 2256 (ATCC No. 13869), which had been cultured for 15 hours. Fermentation was carried out in the jar at 31°C under aeration and agitation. During fermentation, gaseous ammonia was introduced into the jar together with air whereby the pH value of the culture medium was kept at approximately 7.8. 48 hours after inoculation, fermentation was completed and the concentration of L-glutamic acid in the fermentation broth was 4.52 g/dl. The broth was filtered after adding 1.5 percent of diatomaceous earth to remove the bacterial cells. The filtrate was decolourised by the addition of activated carbon, and finally concentrated under reduced pressure. When the residue was acidified to pH 3.2 with hydrochloric acid and cooled overnight in an ice box, 531 grams of crude L-glutamic acid were precipitated and recovered by filtration.

Example 9

100 ml of raw beet juice (extracted sugar juice from beet) were treated with water and the concentration of sugar in the solution was adjusted to 10% weight/volume. A culture medium was prepared from the diluted solution by adding 0.2% weight/volume of potassium dihydrogen phosphate, 0.04% weight/volume of magnesium sulphate heptahydrate and 0.05% weight/volume of the surface active agent Tween 40. 20 ml of batches of the culture medium were placed in 500 ml flasks, sterilised at 120°C for 20 minutes, and inoculated with seed liquor of *Brevibacterium lactofermentum*, Strain No. 2256 (ATCC No. 13869), which had been cultured previously, in a concentration of 5% weight/volume. The fermentation was allowed to proceed at 31°C for 44 hours while shaking and a 45% weight/volume aqueous solution of urea was added to each flask during fermentation to keep the culture medium slightly alkaline. The medium after fermentation contained 4.15 g/dl L-glutamic acid. When the fermentation was allowed to proceed in the same manner as above but using the medium which did not contain Tween 40, the concentration of L-glutamic acid in the culture medium was 1.06 g/dl.

Example 10

Blackstrap molasses was dissolved in water and diatomaceous earth was added to this solution, which was thereafter suction filtered. A culture medium was prepared from

6.6 litres of the clarified solution, 14 grams of potassium dihydrogen phosphate, 5.6 grams of magnesium sulphate heptahydrate and 56 grams of the surface active agent Tween 40. The pH of medium was adjusted to 7.0, diluted to 13.3 litres and placed in a 20 litre stainless steel fermentation jar. The medium in the jar was sterilised at 115°C for 20 minutes, cooled and inoculated with 700 ml of a seed liquor of *Brevibacterium saccharoliticum*, (ATCC No. 14066), which had been cultured 15 hours. Fermentation was carried out in the jar at 31°C under aeration and agitation. During fermentation, gaseous ammonia was introduced into the jar together with air, whereby the pH value of the culture medium was kept at approximately 7.8. 48 hours after inoculation, the fermentation was complete and the concentration of L-glutamic acid in the fermentation broth was 4.13 grams per decilitre.

Example 11

Blackstrap molasses was dissolved in water and diatomaceous earth was added to this solution, which was thereafter suction filtered. A culture medium was prepared from 6.6 litres of the filtered solution, 14 grams of potassium dihydrogen phosphate and 5.6 grams of magnesium sulphate heptahydrate. The pH of medium was adjusted to 7.0, diluted to 13.3 litres and placed in a 20 litre stainless steel fermentation jar. The medium in the jar was sterilised at 115°C for 20 minutes, cooled and inoculated with 700 ml of a seed liquor of *Brevibacterium divaricatum* (NRRL B-2312), which had been cultured for 15 hours. Fermentation was carried out in the jar at 31°C under aeration and agitation. During fermentation, gaseous ammonia together with air was introduced into the jar whereby the pH value of the culture medium was kept at approximately 7.8. When the growth of the microorganism corresponded to a light absorbancy of 0.32 (see the experiment of the Table 2) five hours after inoculation, 84 grams of a surface active agent (Stearyl monoglyceride) were added to the medium. Fermentation was continued for 54 hours. The concentration of L-glutamic acid in fermentation liquid was 3.97 grams per decilitre.

Example 12

First cane molasses were dissolved in water and diatomaceous earth was added. The solution was thereafter suction filtered. A culture medium was prepared from 6.6 litres of filtered solution, 14 grams of potassium dihydrogen phosphate and 5.6 grams of magnesium sulphate heptahydrate. The pH of medium was adjusted to 7.0, diluted to 13.3 litres and placed in a 20 litre stainless steel fermentation jar. The medium in the jar was sterilised at 115°C for 20 minutes, cooled and inoculated with 700 ml of a seed liquor of *Brevibacterium roseum*, (ATCC No.

13825), which had been cultured for 15 hours. Fermentation was carried out in the jar at 31°C under aeration and agitation. During fermentation, gaseous ammonia together with air was introduced into the jar, whereby the pH value of the culture medium was kept at approximately 7.8. When the growth of the microorganism corresponded to a light absorbance of 0.27, 60 grams of the surface active agent Tween 60 were added to medium and fermentation was continued for 42 hours. A total of 645 grams of L-glutamic acid was obtained.

Example 13

Crystals of raw cane sugar were dissolved in water, and diatomaceous earth was added to the solution which was then filtered. The filtrate was diluted with water to a sucrose concentration of 10 g/dl, then a culture medium was prepared from the prepared sugar solution by the addition of 0.2 g/dl of diammonium hydrogen phosphate, 0.04 g/dl of magnesium sulphate heptahydrate, 0.6 g/dl of urea and 0.03 g/dl of the surface active agent Nitto Ester 1015 (a Trade name of Sucrose monostearate). The pH of the medium was adjusted to 7.0 with aqueous ammonia solution. 20 ml batches of the culture medium were placed in 500 ml flasks, sterilised and inoculated with *Brevibacterium immariophilium* (ATCC No. 14068), which had previously been cultured. The fermentation was allowed to proceed at 28-30°C for 42 hours while shaking. An aqueous solution of urea was added to each flask during fermentation to keep the culture medium at pH 7.5 - 8.2. The concentration of L-glutamic acid in the fermentation broth was 3.24 g/dl.

Example 14

Beet molasses containing 67 g/dl of sucrose was diluted with water to approximately twice its initial volume, and diatomaceous earth was added to the diluted molasses which was then filtered. The filtrate was diluted with water to a sucrose concentration of 10 g/dl, then a culture medium was prepared from the sugar solution by the addition of 0.1 g/dl of potassium dihydrogen phosphate, 0.04 g/dl of magnesium sulphate heptahydrate, and 0.6 g/dl of urea. The pH of the medium was adjusted to 7.0 with aqueous ammonia solution. 20 ml batches of the culture medium were placed in 500 ml flasks, sterilised at 120°C for 15 minutes, and inoculated with *Brevibacterium lactofermentum*, Strain No. 2256 (ATCC No. 13869), which had previously been cultured for 24 hours. The fermentation was allowed to proceed at 37°C while shaking. A 45% weight/volume aqueous solution of urea was added to each flask during the fermentation to keep the culture medium slightly alkaline. 6.5 hours after inoculation, the growth of microorganism corresponded to a light ab-

sorbance of 0.40. Tween 60 was added to fermentation medium in a concentration of 0.03 g/dl, and fermentation was continued for 40 hours. The concentration of L-glutamic acid in fermentation liquid was 4.89 g/dl.

Example 15

Beet molasses containing 67 g/dl of sucrose was diluted with water to approximately twice its initial volume, and diatomaceous earth was added to the diluted molasses which was then filtered. The filtrate was further diluted with water to a sucrose concentration of 10 g/dl, then a culture medium was prepared from the sugar solution by the addition of 0.1 g/dl of potassium dihydrogen phosphate, 0.04 g/dl of magnesium sulphate heptahydrate, and 0.6 g/dl of urea. The pH of the medium was adjusted to 7.0 with aqueous ammonia solution. 20 ml batches of the culture medium were placed in 500 ml flasks, sterilised at 120°C for 15 minutes, and inoculated with *Brevibacterium lactofermentum*, Strain No. 2256 (ATCC No. 13869), which had previously been cultured for 24 hours. The fermentation was allowed to proceed at 30°C while shaking. A 45% weight/volume aqueous solution of urea was added to each flask during fermentation to keep the culture medium at pH 7.7. 7 hours after inoculation, the growth of the microorganism corresponded to a light absorbance of 0.41. Polyoxyethylene palmitate was then added to fermentation medium in a concentration of 0.12 g/dl, and fermentation was continued for 52 hours. The concentration of L-glutamic acid in fermentation liquid was 4.31 g/dl.

Example 16

Cane molasses containing 67 g/dl sucrose was diluted with water to approximately twice its initial volume, and diatomaceous earth was added to the diluted molasses which was then filtered. The filtrate was further diluted with water to a sucrose concentration of 10 g/dl, then a culture medium was prepared from the sugar solution by the addition of 0.1 g/dl of potassium dihydrogen phosphate, 0.04 g/dl of magnesium sulphate heptahydrate, and 0.6 g/dl of urea. The pH of the medium was adjusted to 7.0 with aqueous ammonia solution. 20 ml batches of the culture medium were placed in 500 ml flasks, sterilised at 120°C for 15 minutes, and

inoculated with *Brevibacterium lactofermentum*, Strain No. 2256 (ATCC No. 13869), which had previously been cultured for 24 hours. The fermentation was allowed to proceed at 33°C while shaking. A 45% weight/volume aqueous solution of urea was added to each flask during the fermentation to keep the culture medium at pH 7.9. 5.5 hours after inoculation, the growth of the microorganism corresponded to a light absorbance of 0.28. Polyoxyethylene octadecyl ether was then added to fermentation medium in a concentration of 0.44 g/dl, and fermentation was continued for 44 hours. When the fermentation was terminated, the concentration of L-glutamic acid in fermentation liquid was 3.97 g/dl.

WHAT WE CLAIM IS:—

1. A process for producing L-glutamic acid, which comprises cultivating a microorganism capable of producing L-glutamic acid in the presence of beet molasses, cane molasses, thin cane juice, raw cane sugar, raw beet sugar or raw beet juice and in the presence of up to 1% wt/vol., calculated on the volume of the medium, of a surface active agent.
2. A process as claimed in claim 1, wherein the surface active agent is non-ionic.
3. A process as claimed in claim 1 or claim 2, wherein the molasses, juice or sugar consists of ~~cane~~ molasses or beet molasses.
4. A process as claimed in any of the preceding claims, wherein the concentration of the surface active agent is 0.01 to 0.1% wt/vol., calculated on the total volume of the medium.
5. A process for the production of L-glutamic acid, substantially as described with reference to any of Examples 1 to 4.
6. A process for the production of L-glutamic acid, substantially as described with reference to any of Examples 5 to 9.
7. A process for the production of L-glutamic acid, substantially as described with reference to any of Examples 10 to 16.
8. L-glutamic acid when produced by the process claimed in any one of the preceding claims.

ELKINGTON AND FIFE

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sorbance of 0.40. Tween 60 was added to fermentation medium in a concentration of 0.03 g/dl, and fermentation was continued for 40 hours. The concentration of L-glutamic acid in fermentation liquid was 4.89 g/dl.

Example 15

Beet molasses containing 67 g/dl of sucrose was diluted with water to approximately twice its initial volume, and diatomaceous earth was added to the diluted molasses which was then filtered. The filtrate was further diluted with water to a sucrose concentration of 10 g/dl, then a culture medium was prepared from the sugar solution by the addition of 0.1 g/dl of potassium dihydrogen phosphate, 0.04 g/dl of magnesium sulphate heptahydrate, and 0.6 g/dl of urea. The pH of the medium was adjusted to 7.0 with aqueous ammonia solution. 20 ml batches of the culture medium were placed in 500 ml flasks, sterilised at 120°C for 15 minutes, and inoculated with *Brevibacterium lactofermentum*, Strain No. 2256 (ATCC No. 13869), which had previously been cultured for 24 hours. The fermentation was allowed to proceed at 30°C while shaking. A 45% weight/volume aqueous solution of urea was added to each flask during fermentation to keep the culture medium at pH 7.7. 7 hours after inoculation, the growth of the microorganism corresponded to a light absorbance of 0.41. Polyoxyethylene palmitate was then added to fermentation medium in a concentration of 0.12 g/dl, and fermentation was continued for 52 hours. The concentration of L-glutamic acid in fermentation liquid was 4.31 g/dl.

Example 16

Cane molasses containing 67 g/dl sucrose was diluted with water to approximately twice its initial volume, and diatomaceous earth was added to the diluted molasses which was then filtered. The filtrate was further diluted with water to a sucrose concentration of 10 g/dl, then a culture medium was prepared from the sugar solution by the addition of 0.1 g/dl of potassium dihydrogen phosphate, 0.04 g/dl of magnesium sulphate heptahydrate, and 0.6 g/dl of urea. The pH of the medium was adjusted to 7.0 with aqueous ammonia solution. 20 ml batches of the culture medium were placed in 500 ml flasks, sterilised at 120°C for 15 minutes, and

inoculated with *Brevibacterium lactofermentum*, Strain No. 2256 (ATCC No. 13869), which had previously been cultured for 24 hours. The fermentation was allowed to proceed at 33°C while shaking. A 45% weight/volume aqueous solution of urea was added to each flask during the fermentation to keep the culture medium at pH 7.9. 5.5 hours after inoculation, the growth of the microorganism corresponded to a light absorbance of 0.28. Polyoxyethylene octadecyl ether was then added to fermentation medium in a concentration of 0.44 g/dl, and fermentation was continued for 44 hours. When the fermentation was terminated, the concentration of L-glutamic acid in fermentation liquid was 3.97 g/dl.

WHAT WE CLAIM IS:—

1. A process for producing L-glutamic acid, which comprises cultivating a microorganism capable of producing L-glutamic acid in the presence of beet molasses, cane molasses, thin cane juice, raw cane sugar, raw beet sugar or raw beet juice and in the presence of up to 1% wt/vol., calculated on the volume of the medium, of a surface active agent.

2. A process as claimed in claim 1, wherein the surface active agent is non-ionic.

3. A process as claimed in claim 1 or claim 2, wherein the molasses, juice or sugar consists of ~~can~~ molasses or beet molasses.

4. A process as claimed in any of the preceding claims, wherein the concentration of the surface active agent is 0.01 to 0.1% wt/vol., calculated on the total volume of the medium.

5. A process for the production of L-glutamic acid, substantially as described with reference to any of Examples 1 to 4.

6. A process for the production of L-glutamic acid, substantially as described with reference to any of Examples 5 to 9.

7. A process for the production of L-glutamic acid, substantially as described with reference to any of Examples 10 to 16.

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

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