

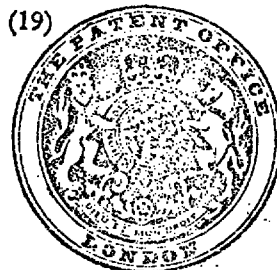
PATENT SPECIFICATION

(11) 1447246

1447246

- (21) Application No. 46803/75 (22) Filed 2 Oct. 1973
 (62) Divided out of No. 1447245
 (31) Convention Application No. 294397 (32) Filed 2 Oct. 1972 in
 (33) United States of America (US)
 (44) Complete Specification published 25 Aug. 1976
 (51) INT CL³ C13K 11/00
 (52) Index at acceptance
 C2S

(19)



(54) PROCESS FOR THE PRODUCTION OF LEVULOSE-BEARING SYRUPS

(71) We, CPC INTERNATIONAL INC., a Corporation organised under the laws of the State of Delaware, United States of America, of International Plaza, Englewood Cliffs, New Jersey 07632, United States of America, 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 generally to enzymatic isomerization. More particularly, the invention relates to improvements in continuous isomerization techniques.

The enzymatic hydrolysis of starch, for the production of corn syrup and corn syrup solids, is routinely conducted on a commercial scale to produce hydrolysates at D.E. values in excess of 95, utilizing what are essentially batch techniques.

The reported investigations of the enzymatic isomerization of dextrose and of starch hydrolysates, for the production of levulose-bearing products, have generally utilized batch isomerization techniques. An exemplary batch isomerization, for example, might be conducted in a stirred tank reactor at a temperature of about 70°C and at a pH of about 6.2, for an isomerization period of from about 40 to 60 hours. The enzyme dosage and conversion temperature are selected to obtain a desired levulose content in the end product. The pH of the syrup would be maintained at about 6.2 by either continuous or incremental additions of a basic material, such as sodium bicarbonate, during isomerization.

The selection of optimum operating conditions, for batch isomerization, is not easy. One initial variable that can be fixed, to some extent, is the particular enzyme preparation that is employed. Isomerase enzymes have their own individual characteristics and those derived from different microorganisms cannot be considered to have the same optimum conditions for action. For example, each enzyme preparation ordinarily will be found to have its own optimum pH, optimum temperature, metal requirement, temperature stability, and

Michaelis constant, among other properties, as is reported, for example, by Dr. T. Sato, in "The Enzyme for the Isomerization of Glucose", in the Japanese publication *Dempunto Gijutsu Kenkyu Kaiho* 32, 81—88 (1965).

When the enzyme preparation is derived from a microorganism of the *Streptomyces* genus, the rate of levulose formation by the enzyme is much greater at 70°C than at 60°C. However, at pH 6.2, some of the advantages of conducting the isomerization at the higher temperature are lost, since the slightly acid pH reduces the rate of levulose formation and reduces enzyme stability. In fact, neither the 70°C operating temperature nor the slightly acidic pH are conducive to enzyme stability.

For effective batch isomerization, continuous mixing of the starch hydrolysate solution (syrup) is necessary. Unfortunately, when cells are used as the source of enzyme activity, the mixing action apparently imposes shear forces on the microbial cells that contain the enzyme. When cells are ruptured, the cellular fluids, including the enzyme, are released into the solution. The enzyme appears to be more susceptible to inactivation under these conditions, than enzyme that remains within intact cells.

Some consideration of the effect of temperature on isomerase has been reported in the literature. For example, two of the pioneer workers in the field, N. Tsumura and T. Sato of the Japanese Food Research Institute, published a series of papers entitled, "Enzymatic Conversion of D-Glucose to D-Fructose". In parts V and VI, which appear in *Agr. Biol. Chem.* 29, 1123—1128 and 1129—1134, respectively (1965), they describe the effects of temperature on an isomerase obtained from *Aerobacter cloacae*. They found that this enzyme lost the majority of its activity at 80°C in the presence of magnesium and at 90°C in the presence of cobaltous ion. Without the presence of stabilizing metal ions, exposure of their enzyme to a temperature of 70°C for a ten-minute period resulted in

50

55

60

65

70

75

80

85

90

95

very substantial loss of enzyme activity. The addition of stabilizing metal ions enhanced heat tolerance, but even so, enzyme inactivation could be observed at about 80°C.

- 5 An article by Yoshimura, Danno and Nataka, of Hyogo University, Japan, appearing in *Agr. Biol. Chem.* 30 (10), 1015-1023 (1966), entitled "Studies on Glucose Isomerizing Activity of D-xylose Grown Cells from *Bacillus coagulans*, Strain HN-68", reports the effect of temperature on the isomerase enzyme from their source microorganism. Maximum activity after two hours incubation was observed at 70°C. Maximum enzyme activity after twenty hours incubation was observed at temperatures in the range from 60°C to 65°C. Above 70°C, substantial inactivation of the enzyme could be observed after two hours, and when operating temperatures for given enzyme dose batches were compared after twenty hours, substantial enzyme inactivation appeared to occur above 65°C.

- 15 In the *Journal of Food Science and Technology* (Nippon Shokuhin Kogei Gakkaishi) 14 (12), 539-540 (1967), N. Tsumura and M. Ishikawa, in "Continuous Isomerization of Glucose by a Column of Glucose Isomerase", described a continuous isomerization technique in which a purified isomerase enzyme sample, derived from a strain of *Streptomyces*, was anchored on a DEAE-Sephadex ("Sephadex" is a Registered Trade Mark) bed in a column, to immobilize the enzyme. A glucose solution was then passed through the column. The column was heated by warm water at 60°C, which was passed through a jacket. An eventual reduction in enzyme activity was observed, although the authors were not certain whether the decrease in observed isomerization was caused by enzyme inactivation or by leaching of the enzyme from the column.

- 25 More recently, in a paper appearing in the text, "Fermentation Advances", edited by D. Perlman, Academic Press, 1969, beginning at page 561, Dr. Takasaki and his associates from the Japanese Fermentation Research Institute have described a continuous column isomerization and have characterized the heat stability of an isomerase enzyme derived from a particular strain of *Streptomyces*. They observed enzyme inactivation after ten minutes at temperatures above 70°C.

- 30 One object of the present invention is to provide new and improved practical techniques for conducting the enzymatic isomerization of dextrose to levulose on a continuous basis.

- 35 Another object of the invention is to provide practical processes for the isomerization of starch hydrolysates or dextrose solutions to levulose-bearing products, that are more attractive for commercial exploitation than prior art processes.

Because of the plethora of terms that are in common use in the art, a few definitions are made to simplify the present specification and permit it to be more concise.

D.E.:

The term "D.E." is an abbreviation for "dextrose equivalent", and these terms are used interchangeably to refer to the reducing sugar content of a material calculated as dextrose and expressed as percent of total solids.

Starch Hydrolysate:

The term "starch hydrolysate" is used in a general way to refer to a syrup or dry product that is made by the hydrolysis of starch. Such a product may be made by acid or enzymatic hydrolysis, or by a combination of acid and enzymatic hydrolysis. A preferred type of starch hydrolysate for use for isomerization in accordance with the present invention is produced by acid or enzyme thinning to a D.E. of 10 or less, followed by enzymatic saccharification to a D.E. of at least 95, and preferably above 97.5.

Glucose and Dextrose:

Medium D.E. starch hydrolysates are commonly referred to in the art as "glucose", whether the starch hydrolysate is in the form of a syrup or in the form of solids. The term "dextrose" is commonly reserved for the refined crystalline monosaccharide that is recovered from a high D.E. starch hydrolysate, or for D-glucose as a constituent of starch hydrolysates. As used hereafter, the term "dextrose" will be used to embrace this monosaccharide in any form, in solution or dry, as a constituent of a starch hydrolysate syrup, syrup solids, or in refined crystalline form.

Fructose and Levulose:

The terms "fructose" and "levulose" are generally employed interchangeably in the art to refer to a particular isomer of dextrose that is sweeter than dextrose. This isomer is found in honey and in invert sugar, along with dextrose, and it is valuable because of its sweetness. The term "levulose" will be used to refer to this monosaccharide.

The enzyme:

The enzyme that isomerizes dextrose to levulose has been referred to in the art by several names. It is referred to in the Marshall patent, 2,950,228, as xylose isomerase, because it isomerizes xylose to xylulose. This activity is in addition to its ability to isomerize dextrose to levulose. It has also been referred to in the art as dextrose isomerase and glucose isomerase. The term "xylose isomerase" will be used herein, since investigations have revealed that xylose is the natural substrate of the isomerase.

Enzyme preparation:

The term "enzyme preparation" is used to refer to any composition of matter that exhibits the desired xylose isomerase enzymatic activity. The term is used to refer, for example, to live whole cells, dried cells, cell extracts, and refined and concentrated preparations derived from the cells. Enzyme preparations may be either in dry or liquid form. Since this invention is concerned with continuous processes, the enzyme preparation will always be employed in some immobilized form. For example, the enzyme preparation may be dispersed in a starch hydrolysate solution, but may be retained in that solution, while desired end products are selectively permitted to discharge from the solution through a selective membrane by ultrafiltration techniques. Alternatively, the enzyme preparation may be bound to an insoluble matrix. The three principal methods for binding enzymes to matrices are by ordinary covalent chemical linkages, by adsorption, and by entrapment of the enzyme within a gel lattice having pores large enough to allow the molecules of the substrate and of the product to pass freely, but small enough to retain the enzyme.

Units:

All parts and percentages are by weight, and on as is basis, unless expressly stated to be otherwise.

Isomerase unit:

One isomerase unit is defined as the amount of enzyme activity that is required to produce one micromole of levulose per minute under the isomerization conditions described hereafter under the heading, "Assay of Isomerase Activity".

Streptomyces:

This term refers to a genus of microorganisms of the order of *Actinomycetales*. These microorganisms are aerial mycelium-producing actinomycetes. The genus is well recognised. Some of its important distinguishing characteristics are described, for example, in the text, "The Actinomycetes", by Selman A. Waksman, The Ronald Press Company, New York, 1967, page 135 *et seq.*

The present invention provides a continuous process for the enzymatic isomerization of dextrose to levulose which process comprises

1. an initial process phase in which a continuous stream of a solution of dextrose is passed into contact with a xylose isomerase enzyme preparation, as hereinbefore defined, at a temperature not less than 50°C. and collecting the effluent solution and recovering

levulose therefrom until a decrease in enzyme activity becomes apparent;

2. subsequently increasing the temperature at which the solution of dextrose makes contact with the xylose isomerase enzyme preparation by at least 5°C. such that the activity of the enzyme is enhanced; and

3. a second process phase in which the process is continued at the higher temperature.

Preferably in the first step the temperature does not exceed 70°C. and in the third step 80°C.

In greater detail, in one preferred mode for practising the invention, the process involves subjecting a stream of a solution containing dextrose to the action of an immobilised enzyme preparation at a pH of at least 7.0 and preferably in the range from pH 7.5 to 8.5, and at a temperature level of at least 50°C. but that is at least 10°C. below the temperature at which rapid inactivation of the enzyme occurs, during an initial isomerisation phase. For enzyme preparations derived from microorganisms of the *Streptomyces* genus, ordinarily the temperature above which rapid inactivation of the enzyme preparation can be observed is about 70°C., so that the preferred operating temperature during the initial phase of isomerization is about 60°C or lower, that is, preferably 50 to 65°C more preferably 50°C to 60°C. At temperatures in the range from 50°C to 60°C, some enzyme inactivation occurs, but the rate of inactivation is low relative to the rate of inactivation at 70°C. After some material loss of enzyme activity is observed after operating in the range from 50°C to 60°C or so, the temperature is raised at least about 5°C, either gradually, in increments of 2°—3°C or less, as needed to maintain the desired ketose level in the product, or alternatively, the temperature may be raised at least 5°C in a single step. Preferably, however, the increase in temperature for the second phase of operation is at least about 10°C, preferably through gradual or incremental increases in operating temperature.

Among the objectives and advantages of the present invention are better enzyme stability, more desirable carbohydrate content of the isomerized product, improved efficiency as compared to batch conversions, and operating economy.

Assay of Isomerase Activity

The assay procedure involves making a spectrophotometric determination of the ketose produced from a glucose solution under a standardized set of conditions.

A stock solution is made up in the following manner:

TABLE 1
Stock Solution for Assay

Component	Amount
0.1 M $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	1 ml.
5 0.01 M $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$	1 ml.
1 M Phosphate buffer, pH 7.5	0.5 ml.
Anhydrous D-glucose	1.44 grams
10 Distilled water	To make up a total volume of 7.5 ml.

The enzyme preparation to be assayed is first sonicated or otherwise treated as necessary to put the enzyme in a form suitable for assay and then is diluted to contain from 1 to 15 isomerase units per ml.

An enzymatic isomerization is conducted by adding 1 ml. of the enzyme preparation to 3 ml. of the stock solution, and incubating for 30 minutes at 60°C. At the end of the 20 incubation period, a 1 ml. aliquot is taken and quenched in a 9 ml. volume of 0.5 N perchloric acid. The quenched aliquot is then diluted to a total volume of 250 ml. As a control, for comparative purposes, a glucose 25 blank is also run by substituting 1 ml. of water for the 1 ml. of the enzyme preparation in solution form, at the beginning of the incubation period.

The ketose is then determined by a 30 cysteine-sulphuric acid method. For the purposes of this assay, one isomerase unit is defined as the amount of enzyme activity that is required to produce one micromole of levulose per minute under the isomerization 35 conditions described.

The invention will now be illustrated by the following Examples.

EXAMPLE 1

40 Continuous Isomerization Utilizing a Fixed Bed of Microbial Cells:
Single Step Temperature Adjustment

For this demonstration of the invention, a 45 jacketed column was used. The jacket was connected to a source of hot water, for controlling column temperature during isomerization.

50 A strain of a microorganism of the *Streptomyces* genus, that is recognized as a good isomerase producer, was grown under submerged, aerobic conditions on a medium containing xylose, to produce intracellular isomerase.

After fermentation, magnesium hydroxide was added to the fermenter broth in the ratio of two parts by weight of magnesium hydroxide for each one part by weight of the cell mass in the broth. The slurry thus obtained was filtered, and the filter cake was then dried in an open pan at room temperature. The activity of the dry enzyme preparation thus obtained was 330 units per gram. 55 60

The dry enzyme preparation was dispersed in a 50% W/V solution of dextrose. The slurry was then placed in the jacketed column, and as the slurry was added to the column, small glass beads, approximately 3 mm. in diameter, were added simultaneously. The glass beads served as a support and also prevented the enzyme preparation from packing and thus plugging up the column. In this fashion, approximately 750 units of the enzyme were charged to the column. 65 70

A dextrose syrup at 50% W/V concentration was adjusted to a pH in the range from 7.0 to 7.5 by adding magnesium hydroxide. The syrup was sparged with nitrogen, and was then fed to the top of the column under a nitrogen atmosphere. 75

The isomerized product was collected in aliquots of 15 ml., in test tubes. The test tubes each contained about 5 ml. of 0.5 N perchloric acid to inactivate any soluble isomerase that might be present in the product. 80

The temperature of the column was maintained at about 60°C during an initial phase of operation. The flow rate of dextrose solution through the column was maintained at a substantially uniform rate, and the ketose content of the effluent was 40% to 50% on a dry solids basis. 85 90

The isomerization was conducted in this manner for nine days before a substantial decrease in enzyme activity became apparent as evidenced by a dropping off in the ketose value observed in the effluent. During that initial phase of operation, the average ketose content of the effluent was 38.7%. 95

At the end of this initial phase of operation, the temperature of the column was increased (from the initial level of 60°C) to 70°C in a single step. Isomerization was then continued, at the increased temperature, for an additional period of 24 hours. The ketose content of the product averaged out at about 49%, for the second phase of operation. The results are summarized below in Table 2. 100 105

TABLE 2

Continuous Isomerization:
Single Step Temperature Adjustment

5	Time Days	Temperature °C	Throughput Bed Volumes per hour	Output of approximately 42% d.b. Levulose Product (lbs. d.s. per cu. ft. of bed)	
				Expected at 60°C	Obtained
10	1	60	1.27	951	951
	2	60	1.27	951	951
	3	60	1.27	951	951
	4	60	1.27	951	951
15	5	60	1.25	936	936
	6	60	1.17	876	876
	7	60	1.10	823	823
	8	60	1.03	771	771
	9	70	1.65	734	1235
	10	70	1.65	681	1235

When the isomerization was repeated with an enzyme preparation prepared from a mixture of diatomaceous earth with the microbial cells, closely comparable results were obtained.

EXAMPLE 2

Continuous Isomerization Utilizing a
Fixed Bed: Small Step Temperature Adjustments

Intracellular isomerase was produced by growing a *Streptomyces* culture under submerged, aerobic conditions on a xylose-containing medium.

At the end of the fermentation, diatomaceous earth filter aid was added to the fermentation liquor in the ratio of 2 parts of the filter aid to 1 part of the microorganism cells, dry cell weight. The cell-diatomaceous earth mixture was separated by filtration, washed, and dried, for use as the enzyme preparation.

This enzyme preparation was suspended in dextrose solution and fed into a jacketed column. The lower outlet of the column was connected to a vacuum source to obtain a compact bed. When filled, the column contained 22 lbs. (dry weight) of the enzyme preparation (i.e., the cell-filter aid mixture) per cu. ft. of column volume.

Water at 60°C was passed through the jacket of the column. A solution containing 500 grams d.s. per litre of 95 D.E. starch hydrolysate containing 92% dextrose, dry basis, adjusted to pH 7.8 with magnesium hydroxide, was supplied continuously to the column, using pressure to control the flow rate. The flow rate was adjusted to maintain 42% (dry basis) levulose in the effluent from the column.

After 6 days of operation, the output volume rate had been dropped to 66% of the original value in order to maintain the 42% (dry basis) levulose content. Thereafter, the temperature was increased periodically, as shown in the table, to maintain the output rate of 42% levulose product at not less than 66% of the original output rate. As shown in the table, the total output quantity obtained in a period of 14 days by means of the increase in temperature was 41% greater than the total output which would have been obtained had the temperature been held constant at 60°C.

TABLE 3

Fixed Bed Isomerization with Small Step Temperature Increases

3	Days	Temperature °C	Output of 42% d.b. Levulose Product (lbs. d.s. per cu. ft. of bed)	
			Expected at 60°C	Obtained
5	1	60	787	787
	2	60	787	787
	3	60	787	787
	4	60	787	787
10	5	60	622	622
	6	60	516	516
	7	62	418	590
	8	62	343	516
	9	62	286	516
15	10	64	233	509
	11	64	187	509
	12	66	158	473
	13	68	127	674
	14	70	106	622
20			Total 6144	8695

EXAMPLE 3

Batch Isomerization Compared to
Immobilized Fixed Bed
Continuous Isomerization

25 A fermentation is conducted with a micro-
organism of the *Streptomyces* genus, in a cul-
ture medium containing xylose, to produce
isomerase. The cells are recovered from the
fermentation broth by adding diatomaceous
30 earth to the fermentation broth and filtering.

A portion of the wet filter cake is utilized
as an enzyme preparation for conducting a
batch isomerization with a 95 D.E. corn
starch hydrolysate. Residence time in the
35 stirred reactor is 50 hours at pH 6.25. The

batch isomerization is to provide a compara-
tive basis for the continuous isomerization.

A separate portion of the same enzyme pre-
paration is also employed as a fixed bed
enzyme source, for conducting a continuous
isomerization. Average residence time in the
column is between one and two hours, at pH
8.

The batch isomerization is conducted at
70°C. The continuous isomerization is con-
ducted at 60°C over an initial period of time,
until substantial inactivation of the enzyme is
observed, whereupon the column tempera-
ture is raised to 70°C.

The results are as reported in Table 4, 50
below.

TABLE 4

Batch vs. Column Isomerization of a 95 D.E. Hydrolysate

55	Batch, 50 hrs., pH 6.5		Feed	Isomerisate	Difference
	Ash, % d.b.		1.0	1.8	0.8
	Org. Acid, % d.b.		0.05	0.08	0.03
	Colour		4	200	196
60	Column, 1—2 hrs., pH 8		Feed	Isomerisate	Difference
	Ash, % d.b.		0.5	0.6	0.1
	Org. Acid, % d.b.		0.03	0.04	0.01
	Colour		4	11	7

The batch isomerization is conducted at
pH 6.25 because at pH values above 7.0,
during the long residence time that is required
for the batch process, the carbohydrate com-
position of the end product tends to be some-
what undesirable. The conditions employed
for this comparison are believed to be reason-
ably close to the optimum for each type of

process, and the comparison is highly favour-
able to the continuous process.

In terms of enzyme economy, the con-
tinuous process is much more efficient and
involves the usage of a much lower amount
of enzyme activity per unit of levulose pro-
duct produced.

The invention can also be practised suc-

cessfully with enzyme preparations of a different kind, although generally, the use of intact cells is preferred. Thus, in another demonstration of the invention, dried intact cells are converted to fine powder in a Waring blender. A buffer solution is prepared by dissolving a small amount of the surfactant Tween 80 in a 1.0 M glycine solution ("Tween" is a Registered Trade Mark). The buffer solution is adjusted to pH 8.0 by adding a small amount of sodium hydroxide. A slurry is then prepared by adding 200 grams of the dry, fine powdered cells to 1,000 ml. of the buffer solution. The slurry is then filtered. 793 ml. of filtrate is recovered, having an activity of 17.5 units per ml.

One part of this enzyme solution is then anchored on a porous glass carrier having a high surface area and reactive silanol groups, as described in U.S. Patent No. 3,556,945, granted January 19, 1971. A second portion of the solution is absorbed on activated charcoal granules. Both forms of the enzyme can then be employed in practicing continuous isomerization in accordance with the invention, utilizing the general techniques described above.

One of the most important advantages of the practice of the present invention is lower enzyme cost per unit of levulose produced. As compared to batch isomerization, the same amount of enzyme, when utilized in accordance with the present invention, produces more levulose product than batch processing. Up to three times more product has been produced from a given amount of enzyme preparation, by practicing the present invention, than is obtained when optimum processing batch techniques are employed.

In addition, there are many other important advantages. Generally speaking, continuous isomerisation techniques utilising an immobilised enzyme preparation permit much shorter isomerisation times or residence times, that is, times of contact between the supply solution and the enzyme preparation. This means that the sugars, that are being processed, are subjected to the elevated processing temperature for a shorter time, and there is less thermal degradation. For these reasons, the finished product contains fewer colour bodies, so that product colour is superior and refining costs are lower. In addition, fewer organic acids are formed. Moreover, the carbohydrate composition of the end product is more desirable. Also because of the shorter processing time, smaller inventories are required in process.

Our co-pending Application No. 46081/73 (Serial No. 1447245), relates *inter-alia* to a particularly advantageous form of enzyme comprising xylose isomerase sorbed on basic magnesium carbonate. The enzyme described in our Application No. 46081/73 (Serial No. 1,447,245) can, of course, be used in the

process of the present invention, but no claim is made herein for the subject matter claimed in Application No. 46081/73 (Serial No. 1,447,245).

WHAT WE CLAIM IS:—

1. A continuous process for the enzymatic isomerisation of dextrose to levulose which process comprises

1. an initial process phase in which a continuous stream of a solution of dextrose is passed into contact with a xylose isomerase enzyme preparation, as hereinbefore defined, at a temperature not less than 50°C. and collecting the effluent solution and recovering levulose therefrom until a decrease in enzyme activity becomes apparent;
2. subsequently increasing the temperature at which the solution of dextrose makes contact with the xylose isomerase enzyme preparation by at least 5°C. such that the activity of the enzyme is enhanced; and
3. a second process phase in which the process is continued at the higher temperature.

2. A process as claimed in claim 1 wherein in the second process phase the higher temperature is selected so as to maintain a predetermined level of enzyme activity.

3. A process as claimed in either claim 1 or claim 2 wherein the temperature during the initial process phase is not greater than 70°C.

4. A process as claimed in claim 4 wherein the temperature during the initial process phase is from 50°C. to 65°C.

5. A process as claimed in any one of claims 1 to 4 wherein in the second step the temperature is increased gradually or in a series of increments.

6. A process as claimed in any one of claims 1 to 4 wherein in the second step the temperature is increased in a single increment.

7. A process as claimed in any one of claims 1 to 6 wherein in the second step the temperature is increased by at least 10°C.

8. A process as claimed in any one of claims 1 to 7 wherein in the second phase the temperature does not exceed 80°C.

9. A process as claimed in any one of claims 1 to 8 wherein the solution containing dextrose is a starch hydrolysate.

10. A process as claimed in claim 9 wherein the starch hydrolysate is a corn starch hydrolysate.

11. A process as claimed in either claim 9 or claim 10 wherein the starch hydrolysate has a D.E. (dextrose equivalent) of at least 95.

12. A process as claimed in any one of



cessfully with enzyme preparations of a different kind, although generally, the use of intact cells is preferred. Thus, in another demonstration of the invention, dried intact cells are converted to fine powder in a Waring blender. A buffer solution is prepared by dissolving a small amount of the surfactant Tween 80 in a 1.0 M glycine solution ("Tween" is a Registered Trade Mark). The buffer solution is adjusted to pH 8.0 by adding a small amount of sodium hydroxide. A slurry is then prepared by adding 200 grams of the dry, fine powdered cells to 1,000 ml. of the buffer solution. The slurry is then filtered. 793 ml. of filtrate is recovered, having an activity of 17.5 units per ml.

One part of this enzyme solution is then anchored on a porous glass carrier having a high surface area and reactive silanol groups, as described in U.S. Patent No. 3,556,945, granted January 19, 1971. A second portion of the solution is absorbed on activated charcoal granules. Both forms of the enzyme can then be employed in practising continuous isomerization in accordance with the invention, utilizing the general techniques described above.

One of the most important advantages of the practice of the present invention is lower enzyme cost per unit of levulose produced. As compared to batch isomerization, the same amount of enzyme, when utilized in accordance with the present invention, produces more levulose product than batch processing. Up to three times more product has been produced from a given amount of enzyme preparation, by practising the present invention, than is obtained when optimum processing batch techniques are employed.

In addition, there are many other important advantages. Generally speaking, continuous isomerisation techniques utilising an immobilised enzyme preparation permit much shorter isomerisation times or residence times, that is, times of contact between the supply solution and the enzyme preparation. This means that the sugars, that are being processed, are subjected to the elevated processing temperature for a shorter time, and there is less thermal degradation. For these reasons, the finished product contains fewer colour bodies, so that product colour is superior and refining costs are lower. In addition, fewer organic acids are formed. Moreover, the carbohydrate composition of the end product is more desirable. Also because of the shorter processing time, smaller inventories are required in process.

Our co-pending Application No. 46081/73 (Serial No. 1447245), relates *inter-alia* to a particularly advantageous form of enzyme comprising xylose isomerase sorbed on basic magnesium carbonate. The enzyme described in our Application No. 46081/73 (Serial No. 1,447,245) can, of course, be used in the

process of the present invention, but no claim is made herein for the subject matter claimed in Application No. 46081/73 (Serial No. 1,447,245).

WHAT WE CLAIM IS:—

1. A continuous process for the enzymatic isomerisation of dextrose to levulose which process comprises

1. an initial process phase in which a continuous stream of a solution of dextrose is passed into contact with a xylose isomerase enzyme preparation, as hereinbefore defined, at a temperature not less than 50°C. and collecting the effluent solution and recovering levulose therefrom until a decrease in enzyme activity becomes apparent;
2. subsequently increasing the temperature at which the solution of dextrose makes contact with the xylose isomerase enzyme preparation by at least 5°C. such that the activity of the enzyme is enhanced; and
3. a second process phase in which the process is continued at the higher temperature.

2. A process as claimed in claim 1 wherein in the second process phase the higher temperature is selected so as to maintain a predetermined level of enzyme activity.

3. A process as claimed in either claim 1 or claim 2 wherein the temperature during the initial process phase is not greater than 70°C.

4. A process as claimed in claim 4 wherein the temperature during the initial process phase is from 50°C. to 65°C.

5. A process as claimed in any one of claims 1 to 4 wherein in the second step the temperature is increased gradually or in a series of increments.

6. A process as claimed in any one of claims 1 to 4 wherein in the second step the temperature is increased in a single increment.

7. A process as claimed in any one of claims 1 to 6 wherein in the second step the temperature is increased by at least 10°C.

8. A process as claimed in any one of claims 1 to 7 wherein in the second phase the temperature does not exceed 80°C.

9. A process as claimed in any one of claims 1 to 8 wherein the solution containing dextrose is a starch hydrolysate.

10. A process as claimed in claim 9 wherein the starch hydrolysate is a corn starch hydrolysate.

11. A process as claimed in either claim 9 or claim 10 wherein the starch hydrolysate has a D.E. (dextrose equivalent) of at least 95.

12. A process as claimed in any one of

claims 1 to 8 wherein the solution containing dextrose is prepared by dissolving crystalline dextrose in an aqueous medium.

5 13. A process as claimed in any one of claims 1 to 12 wherein the process is carried out at a pH of at least 7.

14. A process as claimed in claim 13 wherein the pH is maintained at a pH of from 7.5 to 8.5.

10 15. A process as claimed in any one of claims 1 to 14 wherein the xylose enzyme preparation is derived from an organism of the *Streptomyces* genus.

15 16. A process as claimed in any one of claims 1 to 15 wherein the xylose isomerase preparation comprises intact cells of the microorganism.

20 17. A process as claimed in any one of claims 1 to 15 wherein the xylose isomerase enzyme preparation is a cell-free preparation.

18. A process as claimed in any one of claims 1 to 17 wherein the xylose isomerase enzyme preparation is an immobilized preparation.

25 19. A process as claimed in claim 19 wherein the immobilized xylose isomerase preparation is a stabilised enzyme preparation.

30 20. A process as claimed in claim 19 wherein the immobilized and stabilized xylose isomerase preparation comprises xylose isomerase sorbed onto basic magnesium carbonate.

21. A process as claimed in any one of claims 18 to 20 wherein the xylose isomerase preparation is packed into a column.

35 22. A process as claimed in any one of claims 1 to 21 and substantially as hereinbefore described.

23. Levulose whenever made by the process claimed in any one of claims 1 to 22.

STEVENS, HEWLETT & PERKINS,
Chartered Patent Agents,
5, Quality Court,
Chancery Lane,
London, W.C.2.,
Tel.: 01-405 8393.

Printed for Her Majesty's Stationery Office, by the Courier Press, Leamington Spa, 1976.
Published by The Patent Office, 25 Southampton Buildings, London, WC2A 1AY, from
which copies may be obtained.



* C L M S *