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(54) Title: CARBOHYDRATE PROCESS

(57) Abstract

Methods of producing glucosone which comprises enzymatically oxidizing glucose with glucose-2-oxidase in a first zone and separating the concomitantly produced hydrogen peroxide from said first zone through a semi-permeable membrane into a second zone, said membrane being permeable only to compounds of a molecular weight of less than about 100.

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CARBOHYDRATE PROCESS

This invention is concerned with a new and useful process for the production of glucosone and more particularly for the production of glucosone from which food-grade fructose can be obtained.

Commercial methods for the production of fructose, a commercially important sweetner, primarily involve a two-step process, the first, hydrolysis of a polysaccharide such as starch to produce glucose and the second, isomerization of the so-produced glucose to form fructose. The latter step, as is well-known, produces a mixture of glucose and fructose from which it is difficult to separate the desired product, fructose. The commercial separation method involves the use of crystallization techniques which are costly and time-consuming. More detailed description of the various methods of isomerizing glucose can be found in the literature, e.g., U.S. Patent 3,788,945, and 3,616,221.

Glucose can also be converted to fructose by the action of an enzyme, designated glucose-2-oxidase, to form glucosone (D-arabino-2-hexosulose) which in turn can be reduced to fructose with zinc and acetic acid [Folia Microbiol. 23, 292-298 (1978) and Czechoslovakian Patent No. 175897 to Volc et al.].

The reaction of glucose-2-oxidase with glucose to produce glucosone also yields hydrogen peroxide in equimolar amount. The use of the so-produced hydrogen peroxide in the conversion of alkenes to corresponding halohydrins and epoxides has been proposed in European Patent Application 7176. In the published application, the in situ formation of hydrogen peroxide is proposed by inclusion of glucose-2-oxidase and glucose in the reaction mixture which includes a halogenating enzyme and a source of inorganic halide into which the selected alkene is to be introduced. The disclosure of the European patent application further indicates that the glucosone product of the enzymatic oxidation of glucose can be converted to fructose by simple chemical hydrogenation.

However, fructose produced by the said process can be contaminated with significant amounts of by-products from both the enzymatic conversion of glucose and the alkene conversion reaction. In particular, the latter reaction produces halohydrins and alkylene oxides, e.g. ethylene oxide, which are highly toxic materials even at levels in the region of parts per million. Thus, fructose produced by such a process will require careful and costly purification to attain food-grade purity. Further, the potential for contamination of fructose by virtue of secondary reactions during the initial processing stage is quite high due to the highly reactive products, halohydrins and alkyleneoxides, and substantial purification procedures are required to assure the high level purity required for food grade fructose.

SUMMARY OF THE INVENTION

This invention provides a method for the production of glucosone by enzymatic oxidation of glucose to glucosone in a reaction zone from which hydrogen peroxide is removed by use of a hydrogen peroxide-permeable membrane into a second reaction zone.

In accordance with one embodiment of the invention, the second reaction zone contains a reducing agent for the hydrogen peroxide which migrates through the semi-permeable membrane. The presence of the reducing agent in the second zone encourages a faster migration of the hydrogen peroxide out of the first zone. Reducing agents for this embodiment are well-known to those skilled in the art and include a variety of systems such as organic reducing agents, anions, cations and enzymes. Organic reducing agents are exemplified by aldehydes which are readily oxidized to corresponding carboxylic acids. Reducing anions include, for example, oxalate, sulfite, phosphite and iodide ions. Reducing cations include a wide variety of cations which can exist in variable valence states such as the transition metals Fe, Co, Ni, Cr and the like. Reducing enzymes are readily available from a variety of natural products and include catalase and peroxidase. Catalase is found in yeast, eggs and blood while peroxidase is found in horseradish.

The quantity of reducing agents in the second zone is not critical but it is preferably used at levels to significantly reduce the amount of hydrogen peroxide produced in the first reaction zone. Thus, stoichiometric quantities of reducing agent will assure more complete removal of hydrogen peroxide from the first reaction zone and the use of even excess amounts over the stoichiometric will be practical particularly in those cases where the selected reducing agent is readily available and economical.

Of course, the use of less than stoichiometric quantities of the reducing agent is also within the scope of the invention but will be less efficient.

The membranes employed in the present process are for the purpose of establishing two separate zones and permitting migration of hydrogen peroxide from the first to the second zone. The membranes therefore should be of suitable pore size to selectively permit hydrogen peroxide migration, but to preclude passage of larger molecules in the first reaction zone. Such membranes are readily available commercially and can be defined in terms of the molecular weight of solute particles to pass through the membrane. In the present invention, membranes which permit substances of a molecular weight of less than about 100 are to be used, and preferably less than

The migration or passage of hydrogen peroxide through the aforesaid membrane is accomplished through establishment of an equilibrium predicated on the relative concentrations of H_2O_2 on each side of the membrane. As the concentration of hydrogen peroxide in the first zone increases, the H_2O_2 tends to migrate to the second zone until equilibrium is reestablished. The inclusion of a reducing agent in the second zone increases the rate of flow of hydrogen peroxide through the membrane by offsetting the equilibrium in the direction of the second zone, for which reason the reducing agent embodiment is generally preferred.

Employing the present process results in considerable advantage particularly in the further processing of glucosone to fructose. The migration of hydrogen peroxide from the first reaction zone of course affects the rate of the enzymatic oxidation of glucose so that the reaction tends to be more complete and the reaction times can be shorter than normally required. Further, the first reaction zone is essentially free of contaminants that will accumulate primarily in the second reaction zone where the so produced hydrogen peroxide is reacted. The glucosone solution produced in the first reaction zone can be used as such in the hydrogenation step or can be concentrated or otherwise processed as desired. The glucosone solution is substantially free of contaminants other than some unreacted glucose, or glucose dimer or trimer, and whatever contaminants that may have been introduced in the original glucose charge. Usually, the glucose charge will be a hydrolysate of a natural product containing glucose units, most commonly starch, which will contain soluble contaminants such as other carbohydrates, e.g. maltose, formed in the starch hydrolysis.

Accordingly, the reduction of the reaction product of the first zone will provide a product, fructose, which will be comparatively free of contaminants that effect food grade status for the product, the contaminants being derived only from the glucose natural sources, e.g. starches such as corn starch.

PREFERRED EMBODIMENTS

The membranes to be used in the present process are any of those commonly employed in aqueous systems and include a wide variety. Most commonly, the membranes will be comprised of nylon, a styrene polymer, usually polystyrene teflon, or a cellulose ester such as cellulose acetate or propionate. In a first embodiment, the membrane is fitted into a reactor to provide two zones in a manner to preclude unintended mixing of the contents of the two zones. In a second embodiment, separate reactors can be coupled with the selected membrane providing the requisite interface in the coupling. For maximum migration of hydrogen peroxide from the first zone to the second zone, membranes of significant exposed surface area are of course preferred for which reason the first embodiment is more preferable.

The glucose-2-oxidase enzyme can be provided in the form of the enzyme solution in water, immobilized enzyme or immobilized cells or mycelium or the free cells or mycelium. Most commonly since the enzyme is intracellular, the cells or mycelium of the selected microorganism are used by merely suspending them in the reaction solution. Promoters and protectors for the enzyme can also be present. For example, as described in the aforesaid Folia Microbiol. 23, 292-298 (1978), the presence of fluoride ion promotes the enzymatic oxidation of glucose with *O. mucida*. Protectors for enzymes can also be used, e.g. Co, Mn and Mg salts.

The enzymatic oxidation reaction is carried out until substantially complete as can be determined by monitoring the mixture using aliquots to test for glucose content, or by colorimetric determination of glucosone or by determination of of hydrogen peroxide. Usually, reaction periods of about 24-48 hours are sufficent, depending on enzyme potency or activity.

A wide variety of microorganisms can be used to produce the glucose-2-oxidase employed in the present process. For example, the following organisms are described in the literature for this purpose:

I Aspergillus parasiticus [Biochem. J. 31, 1033 (1937)]
II Iridophycus flaccidum [Science 124, 171 (1956)]
III Oudemansiella mucida [Folia Microbiol. 13, 334 (1968) *ibid*. 23, 292-298 (1978)]
IV Gluconobacter roseus [J. Gen Appl. Microbiol. 1,152 (1955)]
V Polyporus obtusus [Biochem. Biophys. Acta 167, 501 (1968)]
VI Corticium caeruleum [Phytochemistry 1977 Vol. 16, p 1895-7]

The temperature for the enzymatic oxidation reaction is not critical. The reaction can be conducted at room temperature, or even somewhat higher than room temperature where the enzyme system employed is of reasonable heat stability. In particular, it is preferable to operate at 50°C. and above. with heat stable enzyme systems in which range bacterial infectio of the reaction mixture is minimized. Alternatively, the enzymatic reaction mixture can contain antibacterial agents to preclude extensive bacterial growth.

The first reaction zone of course should contain no significant amounts of a reducing agent for hydrogen peroxide so that the beneficial results of the present process can be realised. Thus, the system should be substantially free of reducing agents for H_2O_2, i.e. a non reducing system.

During the course of the present process, it is possible for some diffusion of material from the second reaction zone into the first zone, especially where anions, cations or low molecular weight reducing agents are present in the second zone. Therefore, it is usually preferred to use reducing agents which either do not diffuse, e.g. peroxidase and catalase enzymes, particularly in the immobilized form, or which on oxidation form food-acceptable products, e.g. ferric or sulfate which derive from ferrous and sulfite ions on oxidation by H_2O_2. By this preferred procedure, undesirable contamination of the first reaction zone is reasonably avoided.

In a particular preferred form of the invention the separated hydrogen peroxide is reacted with yeast,whole milk or eggs to effect pasteurization at room temperature with the obvious advantage of avoiding elevated temperature normally used for pasteurization. Thus, the yeast, milk and the eggs each are capable of acting as the reducing agent in the second zone.

The reduction of glucosone to fructose is accomplished by known procedures including chemical reduction as with zinc and acetic acid as well as catalytic hydrogenation, with the usual metal catalysts. Of these, the preferred metal catalyst is Raney Ni since its use is compatible with the desired food grade of fructose, i.e. no residues or contaminants are left by this catalyst.

In the ususal procedure employed, the glucosone is hydrogenated at elevated pressure and temperature over the selected metal catalyst until the desired degree of hydrogenation has been achieved. Pressures can range from 100 to 700 atmosphere and even higher while the temperature can range up to about 200°C. Preferred is 100° to 150°C. and a pressure of about 500 atmospheres.

The following example further illustrates the invention.

EXAMPLE

Mycelium of O. mucida are grown in accordance with Example 1 of Czechoslovakian patent 175897 and the equivalent of 15 g. (dry weight) of the mycelium is suspended in 3 L. of 2.5% glucose solution 0.05M NaF in one zone of a 10 L. reactor fitted with a hydrogen peroxide-permeable membrane to form two zones. In the second zone, catalase enzyme immobilized on DEAE-cellulose (Cellex-D, manufactured by Bio-Rad Laboratories) is suspended in 3 L. of water.

The suspension in the first zone is mixed at 25°C. and aerated with oxygen while the second zone is also mixed. After 24 hours the mycelium is then separated from the solution in the first zone and the resulting clear solution is then hydrogenated over Raney Ni at 500 atmospheres hydrogen gas and 100°C. The aqueous mixture is filtered clear of the catalyst, decolorized with carbon, dionized with ion-exchange (anionic and cationic), and concentrated to a fructose syrup at reduced pressure. Alternatively, the aqueous mixture is concentrated and fructose allowed to crystallize.

The fructose obtained as either syrup or crystalline product is of food grade quality.

The membrane employed in this example permits passage of molecules of molecular weight of less than 50.

Essentially the same results are obtained when O. mucida is replaced with the following organisms:

Polyporus obtusus
Radulum casearium
Lenzites Trabea
Irpex flanus
Polyporus versicolor
Pellicularia filamentosa
Armillaria mellea
Schizophyleum commune
Corticium caeruleum

WHAT IS CLAIMED IS:

1. A method of producing glucosone which comprises enzymatically oxidizing glucose with glucose-2-oxidose in a first zone and separating the concomitantly produced hydrogen peroxide from said first zone through a semi-permeable membrane into a second zone, said membrane being permeable only to compounds of a molecular weight of less than about 100.

2. The method according to Claim 1 wherein said second zone comprises a reducing agent for hydrogen peroxide.

3. The method according to Claim 2 wherein said reducing agent is an enzyme.

4. The method according to Claim 3 wherein said enzyme is peroxidase or catalase.

5. The method according to Claim 2 wherein said reducing agent is an anion or cation.

6. The method according to Claim 5 wherein said anion is sulfite.

7. The method according to Claim 5 wherein said cation is ferrous.

8. A method of producing fructose which comprises the steps of

(a) producing glucosone which comprises enzymatically oxidizing glucose with glucose-2-oxidase in a first zone and separating the concomitantly produced hydrogen peroxide from said first zone through a semi-permeable membrane into a second zone, said membrane being permeable only to compounds of a molecular weight of less than about 100, and

(b) reducing the so-produced glucosone to obtain fructose.

9. The method according to Claim 8 wherein the reduction is effected by catalytic hydrogenation.

10. The method according to Claim 9 wherein the catalyst is Raney Ni.

11. The method according to Claim 8 wherein the filtered reaction mixture obtained in Step a is employed in Step b.

INTERNATIONAL SEARCH REPORT

International Application No PCT/US81/00821

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[3]

According to International Patent Classification (IPC) or to both National Classification and IPC

INT. CL^{3} C12P 7/26,19/02; C12N 11/04
U.S. CL. 435/148,105,182

II. FIELDS SEARCHED

Minimum Documentation Searched[4]

Classification System	Classification Symbols
U.S.	435/72,105,147,148,182,190,813,819

Documentation Searched other than Minimum Documentation
to the Extent that such Documents are Included in the Fields Searched[5]

CHEMICAL ABSTRACTS, VOLUMES 76-92(1972-1980)

III. DOCUMENTS CONSIDERED TO BE RELEVANT[14]

Category *	Citation of Document,[16] with indication, where appropriate, of the relevant passages[17]	Relevant to Claim No.[18]
A,P	US,A, 4,246,347, PUBLISHED 20 JANUARY 1981, NEIDLEMAN ET AL.	1-11
A	US,A, 3,911,140, PUBLISHED 07 OCTOBER 1975, OSBORNE ET AL.	1-11
A	ANALYTICAL CHEMISTRY, ISSUED 1965, DOUGLAS A. SKOOG ET AL, HOLT, RINEHART AND WINSTON, NEW YORK, PAGES 319 AND 507-509.	5-7
A,P	US,A, 4,247,641, PUBLISHED 27 JANUARY 1981, NEIDLEMAN ET AL.	1-11
A	CS,A, 175,897, PUBLISHED 27 AUGUST 1976,VOLC ET AL.	1-11

* Special categories of cited documents:[15]

"A" document defining the general state of the art
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IV. CERTIFICATION

Date of the Actual Completion of the International Search[2] 11 SEPTEMBER 1981	Date of Mailing of this International Search Report[2] 06 OCT 1981
International Searching Authority[1] ISA/US	Signature of Authorized Officer[10] Esther M. Kepplinger ESTHER M. KEPPLINGER