either chemically by calcium hydroxide under alkaline conditions or enzymatically by L-arabinose isomerase under pH neutral conditions. The final product is isolated by a combination of filtration and ion exchange chromatography. This process is performed in several tanks or bioreactors. Overall, the method suffers because of the costly separation of other sugars (e.g., D-glucose, D-galactose, and unhydrolyzed lactose) and low product yields. Several methods via microbial cell fermentation are being developed, but none have been proven to be a practical alternative due to their dependence on costly feedstock (e.g., galactitol and D-psicose), low product yields, and costly separation.

[5] There is a need to develop a cost-effective synthetic pathway for high-yield tagatose production where at least one step of the process involves an energetically favorable chemical reaction. Furthermore, there is a need for a tagatose production process where the process steps can be conducted in one tank or bioreactor. There is also a need for a process of tagatose production that can be conducted at a relatively low concentration of phosphate, where phosphate can be recycled, and/or the process does not require using adenosine triphosphate (ATP) as a source of phosphate. There is also a need for a tagatose production pathway that does not require the use of the costly nicotinamide adenosine dinucleotide (NAD(H)) coenzyme in any of the reaction steps.

SUMMARY ~~OF THE INVENTION~~

[6] The ~~inventions~~ disclosure described herein relate to processes for preparing tagatose. In various ~~aspects~~examples, the processes involve converting fructose 6-phosphate (F6P) to tagatose 6-phosphate (T6P), catalyzed by an epimerase; and converting the T6P to tagatose, catalyzed by a phosphatase. The ~~inventions~~disclosure also relate to tagatose prepared by any of the processes described herein.

In one aspect, the disclosure described herein relate to An enzymatic process for preparing tagatose, the process comprising the steps of: (i) converting fructose 6-phosphate (F6P) to tagatose 6-phosphate (T6P) catalyzed by a fructose 6-phosphate epimerase (F6PE); and (II) converting the T6P produced to tagatose catalyzed by a tagatose 6-phosphate phosphatase (T6PP); wherein steps (i)-(ii) are conducted in a single reaction vessel.

[7] In some ~~aspects~~examples of the ~~invention~~disclosure, a process for preparing tagatose also involves the step of converting glucose 6-phosphate (G6P) to the F6P, where the step is catalyzed by phosphoglucose isomerase (PGI). In other ~~aspects~~examples, a process for tagatose synthesis also includes the step of converting glucose 1-phosphate (G1P) to the G6P, and this conversion step is catalyzed by phosphoglucomutase (PGM).

[8] In various ~~aspects~~examples, a process for preparing tagatose can involve converting a saccharide to the G1P, catalyzed by at least one enzyme; converting G1P to G6P, catalyzed by

phosphoglucomutase (PGM); converting G6P to F6P, catalyzed by phosphoglucose isomerase (PGI); converting F6P to tagatose 6-phosphate (T6P), catalyzed by an epimerase; and converting the T6P produced to tagatose, catalyzed by a phosphatase.

[9] The saccharides used in any of the processes can be selected from the group consisting of a starch or its derivative, cellulose or its derivative, and sucrose. The starch or its derivative can be amylose, amylopectin, soluble starch, amylodextrin, maltodextrin, maltose, or glucose. In some ~~aspects~~examples of the ~~invention~~disclosure, a process for preparing tagatose involves converting starch to a starch derivative by enzymatic hydrolysis or by acid hydrolysis of starch. In other ~~aspects~~examples, a starch derivative can be is prepared by enzymatic hydrolysis of starch catalyzed by isoamylase, pullulanase, alpha-amylase, or a combination of two or more of these enzymes. A process for preparing tagatose, in certain ~~aspects~~examples, can also involve adding 4-glucan transferase (4GT).

[10] In various ~~aspects~~examples, a process for preparing tagatose can involve converting fructose to the F6P, catalyzed by at least one enzyme; converting F6P to tagatose 6-phosphate (T6P) catalyzed by an epimerase; and converting the T6P produced to tagatose, catalyzed by a phosphatase. In other ~~embodiments~~examples, a tagatose production process involves converting sucrose to the fructose, catalyzed by at least one enzyme; converting fructose to the F6P, catalyzed by at least one enzyme; converting F6P to tagatose 6-phosphate (T6P) catalyzed by an epimerase; and converting the T6P produced to tagatose, catalyzed by a phosphatase.

[11] In other ~~aspects~~examples of the ~~invention~~disclosure, G6P to be used in a process for preparing tagatose can be generated by converting glucose to the G6P, catalyzed by at least one enzyme. Glucose can in turn be produced by converting sucrose to glucose, catalyzed by at least one enzyme.

[12] In some ~~aspects~~examples of the ~~invention~~disclosure, epimerase used to convert F6P to T6P is fructose 6-phosphate epimerase. The fructose 6-phosphate epimerase can be encoded by a polynucleotide comprising a nucleotide sequence having at least 60%, at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 99%, or 100% sequence identity with SEQ ID NOS.: 1, 3, 5, 7, 9, or 10. In various ~~aspects~~examples, the fructose 6-phosphate epimerase comprises an amino acid sequence having at least 60%, at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 99%, or 100% sequence identity with SEQ ID NOS.: 2, 4, 6, 8, or 11.

[13] In various ~~aspects~~examples of the ~~invention~~disclosure, the phosphatase used to convert T6P to tagatose is tagatose 6-phosphate phosphatase. The tagatose 6-phosphate phosphatase can be encoded by a polynucleotide comprising a nucleotide sequence having at least 60%, at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 99%, or 100% sequence identity with SEQ ID NO.: 12, 14, or 16. In some ~~aspects~~examples of the ~~invention~~disclosure, the tagatose 6-phosphate phosphatase comprises an amino acid sequence having at least 60%, at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 99%, or 100% sequence identity with SEQ ID NO.: 13, 15, or 17.

[14] In various ~~aspects~~examples, a process of the ~~invention~~disclosure are be conducted at a temperature ranging from about 40˚C to about 70˚C, at a pH ranging from about 5.0 to about 8.0, and/or for about 8 hours to about 48 hours. In some ~~aspects~~examples, the steps of a process for preparing tagatose are conducted in one bioreactor. In other ~~aspects~~examples, the steps are conducted in a plurality of bioreactors arranged in series.

[15] In other ~~aspects~~examples of the ~~invention~~disclosure, the steps of a process for preparing tagatose are conducted ATP-free, NAD(H)-free, at a phosphate concentration from about 0 mM to about 150 mM, the phosphate is recycled, and/or at least one step of the process involves an energetically favorable chemical reaction.

BRIEF DESCRIPTION OF THE FIGURES

[16] These drawings illustrate certain aspects of some of the embodiments of the invention, and should not be used to limit or define the invention.

[17] FIG. 1 is a schematic diagram illustrating an enzymatic pathway converting fructose 6-phosphate to tagatose 6-phosphate and then to D-tagatose (tagatose).

[18] FIG. 2 is a schematic diagram illustrating an enzymatic pathway converting starch or its derived products to tagatose. The following abbreviations are used: αGP, alpha-glucan phosphorylase or starch phosphorylase; PGM, phosphoglucomutase; PGI, phosphoglucoisomerase; F6PE, fructose 6-phosphate epimerase; T6PP, tagatose 6-phosphate phosphatase; IA, isoamylase; PA, pullulanase; MP, maltose phosphorylase; PPGK, polyphosphate glucokinase.

[19] FIG. 3 shows an enzymatic pathway converting cellulose or its derived products to tagatose. CDP, cellodextrin phosphorylase; CBP, cellobiose phosphorylase; PPGK,

What is claimed is:

1. An enzymatic process for preparing tagatose, the process comprising the steps of:

(i) converting fructose 6-phosphate (F6P) to tagatose 6-phosphate (T6P) catalyzed by a fructose 6-phosphate ~~an~~ epimerase (F6PE); and

(II) converting the T6P produced to tagatose catalyzed by a tagatose 6-phosphate phosphatase (T6PP);

wherein steps (i)-(ii) are conducted in a single reaction vessel.

~~2. The process of claim 1, further comprising a step of converting glucose 6-phosphate (G6P) to the F6P, wherein the step is catalyzed by phosphoglucose isomerase (PGI).~~

~~3. The process of claim 2, further comprising the step of converting glucose 1-phosphate (G1P) to the G6P, wherein the step is catalyzed by phosphoglucomutase (PGM).~~

~~4. The process of claim 3, further comprising the step of converting a saccharide to the G1P, wherein the step is catalyzed by at least one enzyme, wherein the saccharide is selected from the group consisting of a starch or derivative thereof, cellulose or a derivative thereof and sucrose.~~

~~5. The process of claim 4, wherein at least one enzyme is selected from the group consisting of alpha-glucan phosphorylase (αGP), maltose phosphorylase, sucrose phosphorylase, cellodextrin phosphorylase, cellobiose phosphorylase, and cellulose phosphorylase.~~

~~6. The process of claim 4, wherein the saccharide is starch or a derivative thereof selected from the group consisting of amylose, amylopectin, soluble starch, amylodextrin, maltodextrin, maltose, and glucose.~~

~~7. The process of claim 4 or claim 6, further comprising the step of converting starch to a starch derivative wherein the starch derivative is prepared by enzymatic hydrolysis of starch or by acid hydrolysis of starch.~~

~~8. The process of claim 6, wherein 4-glucan transferase (4GT) is added to the process.~~

~~9. The process of claim 7, wherein the starch derivative is prepared by enzymatic hydrolysis of starch catalyzed by isoamylase, pullulanase, alpha-amylase, or a combination thereof.~~

~~10. The process of claim 1, further comprising:~~

~~the step of converting fructose to the F6P, wherein the step is catalyzed by at least one enzyme; and~~

~~optionally, the step of converting sucrose to the fructose, wherein the step is catalyzed by at least one enzyme.~~

~~11. The process of claim 2, further comprising:~~

~~the step of converting glucose to the G6P, wherein the step is catalyzed by at least one enzyme, and~~

~~optionally, the step of converting sucrose to the glucose, wherein the step is catalyzed by at least one enzyme.~~

~~12. The process of any one of claims 1-11, wherein the epimerase is fructose 6-phosphate epimerase.~~

~~13. The process of claim 12, wherein the fructose 6-phosphate epimerase is encoded by a polynucleotide comprising a nucleotide sequence having at least 60%, at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 99%, or 100% sequence identity with SEQ ID NOS.: 1, 3, 5, 7, 9, or 10.~~

~~14~~2. The process of claim 1~~2~~, wherein the ~~fructose 6-phosphate epimerase~~F6PE comprises an amino acid sequence having ~~at least 60%, at least 70%, at least 80%, at least 85%,~~ at least 90%, at least 95%, at least 97%, at least 99%, or 100% sequence identity with SEQ ID NOS.: 2, 4, 6, 8, or 11.

~~15. The process of any one of claims 1-14, wherein the phosphatase is tagatose 6-phosphate phosphatase.~~

~~16. The process of claim 15, wherein the tagatose 6-phosphate phosphatase is encoded by a polynucleotide comprising a nucleotide sequence having at least 60%, at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 99%, or 100% sequence identity with SEQ ID NOS.: 12, 14, or 16.~~

~~17~~3. The process of claim ~~15~~ 1, wherein the ~~tagatose 6-phosphate phosphatase~~T6PP comprises an amino acid sequence having ~~at least 60%, at least 70%, at least 80%, at least 85%,~~ at least 90%, at least 95%, at least 97%, at least 99%, or 100% sequence identity with SEQ ID NOS.: 13, 15, or 17.

4. The process of claim 2, wherein the T6PP comprises an amino acid sequence having at least 90%, at least 95%, at least 97%, at least 99%, or 100% sequence identity with SEQ ID NOS.: 13, 15, or 17.

~~18. The process of any one of claims 1-17, wherein the process steps are conducted at a temperature ranging from about 40˚C to about 70˚C, at a pH ranging from about 5.0 to about 8.0, and/or for about 8 hours to about 48 hours.~~

~~19. The process of any one of claims 1-18, wherein the process steps are conducted in one bioreactor or in a plurality of bioreactors arranged in series.~~

~~20. The process of any one of claims 1-19, wherein the process steps are conducted ATP-free, NAD(H)-free, at a phosphate concentration from about 0 mM to about 150 mM, the phosphate is recycled, and/or at least one step of the process involves an energetically favorable chemical reaction.~~

~~21. Tagatose prepared by a process of any one of claims 1-20.~~