reaction. Furthermore, there is a need for production process where the process steps can be conducted in one tank or bioreactor. There is also a need for a process of allulose 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 an allulose production pathway that does not require the use of the costly nicotinamide adenosine dinucleotide (NAD(H)) coenzyme in any of the reaction steps.

SUMMARY

[6] The disclosures described herein relate to processes for preparing allulose. In one aspect, the present disclosure refers to a process for preparing allulose, the process comprising: converting fructose 6-phosphate (F6P) to allulose 6-phosphate (A6P), catalyzed by an allulose 6- phosphate epimerase (A6PE); and converting the A6P produced to allulose, catalyzed by an allulose 6-phosphate phosphatase (A6PP). In various examples, the processes involve converting fructose 6-phosphate (F6P) to allulose 6-phosphate (A6P), catalyzed by allulose 6-phosphate 3-epimerase (A6PE); and converting the A6P to allulose, catalyzed by allulose 6-phosphate phosphatase (A6PP). The disclosures also relate to allulose prepared by any of the processes described herein.

[7] In some examples of the disclosure, a process for preparing allulose also involves the step of converting glucose 6-phosphate (G6P) to the F6P, where the step is catalyzed by phosphoglucoisomerase (PGI). In other examples, a process for allulose 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 examples, a process for preparing allulose 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 phosphoglucoisomerase (PGI); converting F6P to allulose 6-phosphate (A6P), catalyzed by A6PE; and converting the A6P produced to allulose, catalyzed by A6PP.

[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 examples of the disclosure, a process for preparing allulose involves converting starch to a starch derivative by enzymatic hydrolysis or by acid hydrolysis of starch. In other examples, a starch derivative can be 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 allulose, in certain examples, can also involve adding 4-glucan transferase (4GT).

[10] In various examples, a process for preparing allulose can involve converting fructose to the F6P, catalyzed by at least one enzyme; converting F6P to allulose 6-phosphate (A6P) catalyzed by A6PE; and converting the A6P produced to allulose, catalyzed by A6PP. In other examples, allulose 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 allulose 6-phosphate (A6P) catalyzed by A6PE; and converting the A6P produced to allulose, catalyzed by A6PP.

[11] In other examples of the disclosure, G6P to be used in a process for preparing allulose 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 other examples of the disclosure, the steps of a process for preparing allulose 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

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

[14] FIG. 1 is a schematic diagram illustrating an enzymatic pathway converting fructose 6-phosphate to allulose 6-phosphate and then to allulose.

[15] FIG. 2 is a schematic diagram illustrating an enzymatic pathway converting starch or its derived products to allulose. The following abbreviations are used: αGP, alpha-glucan phosphorylase or starch phosphorylase; PGM, phosphoglucomutase; PGI,

phosphoglucoisomerase; IA, isoamylase; PA, pullulanase; MP, maltose phosphorylase; PPGK, polyphosphate glucokinase.

[16] FIG. 3 shows an enzymatic pathway converting cellulose or its derived products to allulose. CDP, cellodextrin phosphorylase; CBP, cellobiose phosphorylase; PPGK, polyphosphate glucokinase ; PGM, phosphoglucomutase; PGI, phosphoglucoisomerase.

What is claimed is:

1. A process for preparing allulose, the process comprising:
converting fructose 6-phosphate (F6P) to allulose 6-phosphate (A6P), catalyzed by an allulose 6-phosphate epimerase (A6PE); and
converting the A6P produced to allulose, catalyzed by an allulose 6-phosphate phosphatase (A6PP).

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 a phosphoglucoisomerase (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 a 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 an alpha-glucan phosphorylase (αGP), a maltose phosphorylase, a sucrose phosphorylase, a cellodextrin phosphorylase, a cellobiose phosphorylase, and a cellulose phosphorylase.

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

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

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

9. The process of claim 7, wherein the starch derivative is prepared by the enzymatic hydrolysis of starch catalyzed by an isoamylase, a pullulanase, an 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 the 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 the 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 claim 1, wherein the A6PE comprises an amino acid sequence having at least 90%, at least 95%, or at least 100% sequence identity with the amino acid sequence of SEQ ID NOs.: 3 or 6.

13. The process of claim 1, wherein the A6PP comprises an amino acid sequence having at least 90%, at least 95%, or at least 100% sequence identity with the amino acid sequence of SEQ ID NO.: 9.

14. The process of claim 15, wherein the A6PP is specific for the allulose 6-phosphate.

15. The process of any one of claims 1-11, wherein the process steps are conducted at a temperature ranging from 40˚C to 70˚C, at a pH ranging from 5.0 to 8.0, and/or for 8 hours to about 48 hours.

16. The process of any one of claims 1-11, wherein the process steps are conducted in a plurality of bioreactors arranged in series.

17. The process of any one of claims 1-11, 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.

18. The process of claim 1, wherein the process steps are conducted in one bioreactor.