Summary

[021] The disclosure described herein generally relate to processes for preparing hexoses from saccharides by enzymatic conversion. The disclosure also relate to hexoses prepared by any of the processes described herein.

[0021a] In one aspect, provided herein is a process for preparing mannose from a saccharide, the process comprising: converting fructose 6-phosphate (F6P) to mannose 6-phosphate (M6P) catalyzed by mannose 6-phosphate isomerase (M6PI) or phosphoglucose/phosphomannose isomerase (PGPMI); and converting the M6P to mannose catalyzed by mannose 6-phosphate phosphatase (M6PP).

[022] More specifically, the disclosure relates to processes for preparing a hexose, selected from allose, mannose, galactose, fructose, altrose, talose, sorbose, gulose and idose, from a saccharide, the process comprising: converting fructose 6-phosphate (F6P) to the hexose catalyzed by one or more enzymes selected from an isomerase, an epimerase, and a hexose-specific phosphatase and mixtures thereof.

[023] A process of the disclosure for the production of allose comprises converting the F6P to psicose 6-phosphate (P6P) catalyzed by psicose 6-phosphate 3-epimerase (P6PE); converting the P6P to allose 6-phosphate (A6P) catalyzed by allose 6-phosphate isomerase (A6PI); and converting the A6P to allose catalyzed by allose 6-phosphate phosphatase (A6PP).

[024] A process of the disclosure for the production of mannose comprises converting the F6P to mannose 6-phosphate (M6P) catalyzed by mannose 6-phosphate isomerase (M6PI) or phosphoglucose/phosphomannose isomerase (PGPMI); and converting the M6P to mannose catalyzed by mannose 6-phosphate phosphatase (M6PP).

[025] A process of the disclosure for the production of galactose comprises converting the F6P to tagaose 6-phosphate (T6P) catalyzed by fructose 6-phosphate 4-epimerase (F6PE); converting the T6P to galactose 6-phosphate (Gal6P) catalyzed by galactose 6-phosphate isomerase (Gal6PI); and converting the Gal6P to galactose catalyzed by galactose 6-phosphate phosphatase (Gal6PP).

[026] A process of the disclosure for the production of fructose comprises converting the F6P to fructose catalyzed by fructose 6-phosphate phosphatase (F6PP).

[027] A process of the disclosure for the production of altrose comprises converting the F6P to converting the F6P to P6P catalyzed by P6PE; converting the P6P to altrose 6-phosphate (Alt6P) catalyzed by altrose 6-phosphate isomerase (Alt6PI); and converting the Alt6P produced to altrose catalyzed by altrose 6-phosphate phosphatase (Alt6PP).

[028] A process of the disclosure for the production of talose comprises converting the F6P to T6P catalyzed by F6PE; converting the T6P to talose 6-phosphate (Tal6P) catalyzed by talose 6-phosphate isomerase (Tal6PI); and converting the Tal6P to talose catalyzed by talose 6-phosphate phosphatase (Tal6PP).

[029] A process of the disclosure for the production of sorbose comprises converting the F6P to T6P catalyzed by F6PE; converting the T6P to sorbose 6-phosphate (S6P) catalyzed by sorbose 6-phosphate epimerase (S6PE); and converting the S6P to sorbose catalyzed by sorbose 6-phosphate phosphatase (S6PP).

[030] A process of the disclosure for the production of gulose comprises converting the F6P to T6P catalyzed by F6PE; converting the S6P to gulose 6-phosphate (Gul6P) catalyzed by gulose 6-phosphate isomerase (Gul6PI); and converting the Gul6P to gulose catalyzed by gulose 6-phosphate phosphatase (Gul6PP).

[031] A process of the disclosure for the production of gulose comprises converting the F6P to T6P catalyzed by F6PE; converting the T6P to sorbose 6-phosphate (S6P) catalyzed by sorbose 6-phosphate epimerase (S6PE); converting the S6P to idose 6-phosphate (I6P) catalyzed by idose 6-phosphate isomerase (I6PI); and converting the I6P to idose catalyzed by idose 6-phosphate phosphatase (I6PP).

[032] The processes of hexose production according to the disclosure can involve a step of converting glucose 6-phosphate (G6P) to the F6P, wherein the step is catalyzed by phosphoglucose isomerase (PGI). The processes can also comprise the step of converting glucose 1-phosphate (G1P) to the G6P, wherein the step is catalyzed by phosphoglucomutase (PGM). Additionally, the processes according to the

disclosure may further comprise the step of converting a saccharide to the G1P, where the step is catalyzed by at least one enzyme, and the saccharide is selected from the group consisting of a starch or derivative thereof, cellulose or a derivative thereof, and sucrose.

[033] The enzyme or enzymes used in the step of converting a saccharide to the G1P in the processes according to the disclosure can be alpha-glucan phosphorylase (αGP), maltose phosphorylase, sucrose phosphorylase, cellodextrin phosphorylase, cellobiose phosphorylase, and/or cellulose phosphorylase, and mixtures thereof. When the saccharide is starch or a starch derivative, the derivative may be selected from the group consisting of amylose, amylopectin, soluble starch, amylodextrin, maltodextrin, maltose, and glucose, and mixtures thereof.

[034] Some processes according to the disclosure, may further comprise the step of converting starch to a starch derivative, where the starch derivative is prepared by enzymatic hydrolysis of starch or by acid hydrolysis of starch. Also, 4-glucan transferase (4GT) can be added to the processes. 4GT can be used to increase hexose yields by recycling the degradation products glucose, maltose, and maltotriose into longer maltooligosaccharides; which can be phosphorolytically cleaved by αGP to yield G1P.

[035] Where the processes use a starch derivative, the starch derivative can be prepared by enzymatic hydrolysis of starch catalyzed by isoamylase, pullulanase, alpha-amylase, or their combination.

[036] The process according to the disclosure can also comprise 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.

[037] Furthermore, the processes of producing a hexose according to the disclosure can comprise the step of converting glucose to the G6P, where the step is catalyzed by at least one enzyme, and, optionally, the step of converting sucrose to the glucose that is catalyzed by at least one enzyme.

[038] The steps in each of the processes of hexose synthesis according to the disclosure can be conducted at a temperature ranging from about 40˚C to about 90˚C and at a pH ranging from about 5.0 to about 8.0. They may be conducted for about 8 hours to about 48 hours.

[039] The steps of the processes according to the disclosure can be conducted in a single bioreactor. The steps can also be conducted in a plurality of bioreactors arranged in series.

[040] The enzymatic process steps of the disclosure may be conducted ATP-free and/or NAD(P)(H)-free. The steps can be carried out at a phosphate concentration ranging from about 0.1 mM to about 150 mM. The phosphate used in the phosphorylation and dephosphorylation steps of the processes according to the disclosure can be recycled. At least one step of the processes may involve an energetically favorable chemical reaction.

[041] The disclosure also relates to allose, mannose, galactose, fructose, altrose, talose, sorbose, gulose and idose produced by these processes.

[042] Brief Description of the Drawings

[043] FIG. 1 is a schematic diagram showing an enzymatic pathway converting starch or its derived products to allose. The following abbreviations are used: IA, isoamylase; PA, pullulanase; αGP, alpha-glucan phosphorylase or starch phosphorylase; MP, maltose phosphorylase; PGM, phosphoglucomutase; PPGK, polyphosphate glucokinase; PGI, phosphoglucoisomerase; P6PE, psicose 6-phosphate 3-epimerase; A6PI, allose 6-phosphate isomerase; A6PP, allose 6-phosphate phosphatase.

[044] FIG. 2 is a schematic diagram showing an enzymatic pathway converting starch or its derived products to mannose. The following abbreviations are used: IA, isoamylase; PA, pullulanase; αGP, alpha-glucan phosphorylase or starch phosphorylase; MP, maltose phosphorylase; PGM, phosphoglucomutase; PPGK, polyphosphate glucokinase; PGI, phosphoglucoisomerase; PGPMI,

The claimed invention is:

1. A process for preparing mannose from a saccharide, the process comprising:

converting fructose 6-phosphate (F6P) to mannose 6-phosphate (M6P) catalyzed by mannose 6-phosphate isomerase (M6PI) or phosphoglucose/phosphomannose isomerase (PGPMI); and

converting the M6P to mannose catalyzed by mannose 6-phosphate phosphatase (M6PP).

2. The process of claim 1, further comprising a step of converting glucose 6-phosphate (G6P) to the F6P catalyzed by phosphoglucoisomerase (PGI) or PGPMI.

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

4. The process of claim 3, further comprising the step of converting a saccharide to the G1P catalyzed by at least one enzyme, wherein the saccharide is starch, a starch derivative, or sucrose.

5. The process of claim 4, wherein the at least one enzyme in the step of converting a saccharide to the G1P is selected from the group consisting of alpha-glucan phosphorylase (αGP), a sucrose phosphorylase, and mixtures thereof.

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, and mixtures thereof.

7. The process of 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 4, wherein the starch derivative is prepared by enzymatic hydrolysis of starch catalyzed by an isoamylase, pullulanase, alpha-amylase, or a combination thereof.

10. The process of claim 1, wherein the process steps are conducted at a temperature ranging from 37˚C to 95˚C, at a pH ranging from 5.0 to 8.0, and/or for 3 hours to 48 hours.

11. The process of claim 1, wherein the process steps are conducted in a single bioreactor.

12. The process of claim 1, wherein the process steps are conducted ATP-free, NAD(P)(H)-free, at a phosphate concentration from 0.1 mM to 150 mM, the phosphate is recycled, and/or the catalysis by the hexose-specific phosphatase involves an energetically favorable chemical reaction.

13. The process of claim 1, wherein the M6PI comprises an amino acid sequence having at least 25% sequence identity with any one of SEQ ID Nos: 8-11, and wherein said M6PI catalyzes the conversion of F6P to M6P.

14. The process of claim 13, wherein the M6PI contains two domains with a core of antiparallel β-strands resembling the cupin fold and a third domain consisting of only α-helixes, and a divalent metal cation.

15. The process of claim 1, wherein the PGPMI comprises an amino acid sequence having at least 25% sequence identity with any one of SEQ ID Nos: 15-17, and wherein said PGPMI catalyzes the conversion of F6P to M6P.

16. The process of claim 15, wherein the PGPMI contains two Rossmanoid folds, a GGS motif, a SYSG-X-T-X-ET-hydrophobic motif, an EN signature where the Glu is present for active-site base proton transfer, and an HN signature where the HIS is present for ring opening/closure of the substrate during catalysis.

17. The process of claim 1, wherein the M6PP comprises an amino acid sequence having at least 30% sequence identity with any one of SEQ ID Nos: 12-14, and wherein the phosphatase catalyzes the conversion of M6P to mannose.

18. The process of claim 17, wherein the M6PP contains a Rossmanoid fold domain for catalysis, a C1 capping domain, DxD signature in the 1st β-strand of the Rossmanoid fold, a Thr or Ser at the end of the 2nd β-strand of the Rossmanoid fold, a Lys at the N-terminus of the α-helix C-terminal to the 3rd β-strand of the Rossmanoid fold, and a GDxxxD signature at the end of the 4th β-strand of the Rossmanoid fold.