

CLAIMS

1. A genetically modified microorganism for producing xylitol from xylose comprising:
inducible modification of expression of xylose reductase and
an inducible synthetic metabolic valve comprising:
a gene expression-silencing synthetic metabolic valve characterized by silencing gene expression of one or more genes encoding one or more enzymes; or
an enzymatic degradation synthetic metabolic valve characterized by inducing enzymatic degradation of one or more enzymes, or
a combination thereof.
2. The genetically modified microorganism of claim 1, wherein the xylose reductase is an NADPH dependent xylose reductase.
3. The genetically modified microorganism of claim 1, wherein the xylose reductase is the *xyrA* gene of *A. niger*.
4. The genetically modified microorganism of claim 1, wherein the genetically modified microorganism produces xylitol from a xylose feedstock.
5. The genetically modified microorganism of claim 1, wherein of gene-silencing synthetic metabolic valve or the enzyme degradation synthetic metabolic valve are directed to control of the gene encoding xylose isomerase or the xylose isomerase enzyme.
6. The genetically modified microorganism of claim 1, wherein of gene-silencing synthetic metabolic valve or the enzyme degradation synthetic metabolic valve are directed to control of the gene encoding glucose-6-phosphate dehydrogenase (*zwf*) or the glucose-6-phosphate dehydrogenase (*zwf*) enzyme.
7. The genetically modified microorganism of claim 1, wherein of gene-silencing synthetic metabolic valve or the enzyme degradation synthetic metabolic valve are directed to control of at least two genes, including
a gene encoding glucose-6-phosphate dehydrogenase (*zwf*) or the glucose-6-phosphate dehydrogenase (*zwf*) enzyme; and

a gene encoding xylose isomerase or the xylose isomerase enzyme.

8. The genetically modified microorganism of claim 1, wherein of gene-silencing synthetic metabolic valve or the enzyme degradation synthetic metabolic valve are directed to control of at least two genes, including

a gene encoding glucose-6-phosphate dehydrogenase (*zwf*) or the glucose-6-phosphate dehydrogenase (*zwf*) enzyme; and

a gene encoding enoyl-ACP reductase (*fabI*) or the enoyl-ACP reductase (*fabI*) enzyme.

9. The genetically modified microorganism of claim 1, wherein of gene-silencing synthetic metabolic valve or the enzyme degradation synthetic metabolic valve consist of

silencing of a gene encoding glucose-6-phosphate dehydrogenase (*zwf*)

and enzyme degradation of glucose-6-phosphate dehydrogenase (*zwf*) enzyme; and

enoyl-ACP reductase (*fabI*) enzyme.

10. The genetically modified microorganism of claim 1, wherein expression of xylose reductase, gene expression-silencing synthetic metabolic valve, and the enzymatic degradation synthetic metabolic valve are induced under conditions of a transition phrase of a multi-stage biofermentation process.

11. The genetically modified microorganism of claim 1, wherein the induction occurs via nutrient depletion.

12. The genetically modified microorganism of claim 1, wherein the induction occurs via phosphate depletion.

13. The genetically modified microorganism of claim 1, further comprising a chromosomal deletion.

14. The genetically modified microorganism of claim 1, wherein the silencing of gene expression comprises CRISPR interference and the genetically modified microorganism also expresses a CASCADE guide array, the array comprising two or more genes encoding small guide RNAs each specific for targeting a different gene for simultaneous silencing of multiple genes.

15. The genetically modified microorganism of claim 1, wherein the microorganism produces a xylitol product titer of greater than 20 g/L at about twenty four hours in a biofermentation process.

16. A multi-stage fermentation bioprocess for producing xylitol from a genetically modified microorganism, comprising:

(a) providing a genetically modified microorganism comprising:

a modification of expression of xylose reductase and

a synthetic metabolic valve comprising:

a gene expression-silencing synthetic metabolic valve characterized by silencing gene expression of one or more genes encoding one or more enzymes;

or

an enzymatic degradation synthetic metabolic valve characterized by inducing enzymatic degradation of one or more enzymes, or

a combination thereof.

wherein the one or more enzymes of each synthetic metabolic valve are the same or different; and

(b) growing the genetically modified microorganism in a media with a xylose feedstock;

(c) transitioning from a growth phase to a xylitol producing stage by

inducing the synthetic metabolic valve(s) to slow or stop the growth of the microorganism; and

inducing expression of xylose reductase, thereby

(d) producing xylitol.

17. The multi-stage fermentation bioprocess of claim 16, wherein the gene-silencing synthetic metabolic valve or the enzyme degradation synthetic metabolic valve of the genetically modified microorganism are directed to control of at least two genes, selected from the group consisting of:

a gene encoding glucose-6-phosphate dehydrogenase (*zwf*) or the glucose-6-phosphate dehydrogenase (*zwf*) enzyme;

a gene encoding enoyl-ACP reductase (*fabI*) or the enoyl-ACP reductase (*fabI*) enzyme;

and

a gene encoding xylose isomerase or the xylose isomerase enzyme.

18. The multi-stage fermentation bioprocess of claim 16, wherein the bioprocess

produces a xylitol product titer of greater than 20 g/L at about twenty four hours in a biofermentation process.

19. The multi-stage fermentation bioprocess of claim 16, wherein the inducing transition phrase occurs via phosphate depletion of the growth media.

20. The multi-stage fermentation bioprocess of claim 16, the genetically modified microorganism further comprising a chromosomal deletion.

21. A genetically modified microorganism for producing xylitol, the microorganism comprising:

inducible reduction of xylose isomerase;
inducible reduction of glucose-6-phosphate dehydrogenase activity;
wherein the strain produces xylitol from the feedstock xylose upon induction.

22. The genetically modified microorganism of claim 21, wherein the microorganism is an *E.coli* microorganism.

23. The genetically modified microorganism of claim 21, wherein induction occurs by via nutrient depletion.

24. The genetically modified microorganism of claim 21, wherein induction occurs via phosphate depletion.

25. A multi-stage fermentation bioprocess for producing xylitol from the genetically modified microorganism of claim 21, comprising:

- (a) providing a genetically modified microorganism
- (b) growing the genetically modified microorganism in a media with a xylose feedstock;
- (c) transitioning from a growth phase to a xylitol producing stage by
 - inducing the synthetic metabolic valve(s) to slow or stop the growth of the microorganism; and
 - inducing expression of xylose isomerase, thereby
- (d) producing xylitol.

26. A genetically modified microorganism for producing xylitol, the microorganism comprising:

inducible reduction of xylose reductase;
inducible reduction of glucose-6-phosphate dehydrogenase activity;
inducible reduction of enoyl-ACP reductase;
wherein the strain produces xylitol from the feedstock xylose upon induction.

27. The genetically modified microorganism of claim 26, wherein the microorganism is an *E.coli* microorganism.

28. The genetically modified microorganism of claim 26, wherein induction occurs by via nutrient depletion.

29. The genetically modified microorganism of claim 26, wherein induction occurs via phosphate depletion.

30. A multi-stage fermentation bioprocess for producing xylitol from the genetically modified microorganism of claim 26, comprising:

- (a) providing a genetically modified microorganism
- (b) growing the genetically modified microorganism in a media with a xylose feedstock;
- (c) transitioning from a growth phase to a xylitol producing stage by
 - inducing the synthetic metabolic valve(s) to slow or stop the growth of the microorganism; and
 - inducing expression of xylose reductase, thereby
- (d) producing xylitol.

31. A genetically modified microorganism, in which:
activity of a membrane bound transhydrogenase activity is increased;
activity of a pyruvate ferredoxin oxidoreductase is increased;
activity of a NADPH dependent ferredoxin reductase is increased; and
wherein the microorganism produces at least one chemical product whose biosynthesis requires NADPH.