

CLAIMS

1. A genetically modified *E. coli* microorganism comprising:
 - a production pathway comprising citramalate synthase for the production of citramalate,
 - a conditional induction of SoxS;
 - a conditionally triggered synthetic metabolic valve that silences gene expression of the citrate synthase (*gltA*), or glucose-6-phosphate-dehydrogenase (*zwf*) gene(s); or
 - a conditionally triggered synthetic metabolic valve that enables selective proteolysis of the citrate synthase (*gltA*), or glucose-6-phosphate-dehydrogenase (*zwf*) enzyme(s);
 - wherein the synthetic metabolic valve(s) of the microorganism are conditionally triggered during a stationary phase or non-dividing cellular state;
 - wherein, under conditions of depleting of a limiting nutrient from a growth medium in which the genetically modified microorganism is growing, the stationary phase or non-dividing cellular state is induced;
 - wherein pyruvate-flavodoxin/ferredoxin oxidoreductase enzyme activity is increased within the genetically modified microorganism under aerobic or partially aerobic conditions during the stationary phase or non-dividing cellular state to produce an acetyl CoA pool; and
 - wherein sugar uptake is enhanced within the genetically modified microorganism, when compared to a non-genetically modified microorganism.
2. The genetically modified microorganism of claim 1, wherein the genetically modified microorganism comprises deletion of endogenous *poxB* and *pjlB* genes.
3. The genetically modified microorganism of claim 1, wherein the increased pyruvate-flavodoxin/ferredoxin oxidoreductase enzyme activity is due to overexpression of a gene encoding pyruvate ferredoxin oxidoreductase during the stationary phase or non-dividing cellular state.
4. The genetically modified microorganism of claim 4, wherein the pyruvate-flavodoxin/ferredoxin oxidoreductase enzyme is encoded by the *ydbK*.

5. The genetically modified microorganism of claim 1, wherein the increased pyruvate ferredoxin oxidoreductase enzyme activity is due to induction of oxidative soxRS regulon during the stationary phase or non-dividing cellular state.
6. The genetically modified microorganism of claim 1, wherein the increased pyruvate ferredoxin oxidoreductase enzyme activity is increased as the result of reduced NADPH levels within the genetically modified microorganism during the stationary phase or non-dividing cellular state.
7. The genetically modified microorganism of claim 1, wherein the activity of at least one sugar transporter is increased to enhance sugar uptake during the stationary phase or non-dividing cellular state.
8. The genetically modified microorganism of claim 7, wherein constitutive expression of a sugar transporter gene results in increased sugar transporter activity within the genetically modified microorganism.
9. The genetically modified microorganism of claim 7, wherein the sugar transporter is conditionally overexpressed during the stationary phase or non-dividing cellular state.
10. The genetically modified microorganism of claim 7, wherein the sugar transporter is encoded by a *pts* gene.
11. The genetically modified *E. coli* microorganism of claim 1, wherein the synthetic metabolic valves effect gene silencing by CRISPR interference, synthetic metabolic valves further comprising 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, the guide array comprising more than one promotor for each gene.
12. A bioprocess for production of a protein product from the genetically modified

microorganism of claim 1, the bioprocess comprising:

in a first stage, growing the genetically modified microorganism in a medium and

in a second stage, upon depletion of a limiting nutrient from a growth medium, inducing a stationary phase or non-dividing cellular state,

wherein the genetically modified microorganism in the stationary phase or non-dividing cellular state produces product at a rate of 30 g/L or greater.

13. The bioprocess of claims 12, wherein the increased activity of a pyruvate-flavodoxin/ferredoxin oxidoreductase enzyme is caused by overexpression of a gene encoding an active pyruvate ferredoxin oxidoreductase, induction of oxidative soxRS regulon, reducing NADPH levels, reducing glucose-6-phosphate dehydrogenase levels with a synthetic metabolic valve directed to gene silencing of the *zwf* gene or selective proteolysis of the glucose-6-phosphate dehydrogenase enzyme, the valve activated in the stationary phase or non-dividing cellular state, or a combination thereof.

14. The bioprocess of claim 12, wherein the activity of at least one sugar transporter is increased.

15. The bioprocess of claim 12, wherein the product is citramalate, an enzyme of the production pathway comprises citramalate synthase, and the bioprocess produces citramalate at or greater than 100 g/L.

16. The bioprocess of claim 15, wherein the citramalate synthase enzyme is encoded by the *cimA3.7* gene.

17. The bioprocess of claim 12, wherein the genetically modified microorganism comprises a plasmid comprising a citramalate synthase gene is operably linked to a low phosphate inducible promotor.

18. The bioprocess of claim 12, wherein the genetically modified microorganism comprises

deletion of endogenous *poxB* and *pjlB* genes.

19. A genetically modified microorganism comprising:
- a production pathway comprising at least one enzyme for producing a product from an acetyl CoA precursor, and
 - a conditional induction of SoxS;
 - a conditionally triggered synthetic metabolic valve that silences gene expression of the citrate synthase (*gltA*), or glucose-6-phosphate-dehydrogenase (*zwf*) gene(s); and
 - a conditionally triggered synthetic metabolic valve that enables selective proteolysis of the citrate synthase (*gltA*), or glucose-6-phosphate-dehydrogenase (*zwf*) enzyme(s); and
 - deletion of endogenous *poxB* and *pjlB* genes;
- wherein the synthetic metabolic valve(s) of the microorganism are conditionally triggered during a stationary phase or non-dividing cellular state;
- wherein, under conditions of depleting of a limiting nutrient from a growth medium in which the genetically modified microorganism is growing, the stationary phase or non-dividing cellular state is induced;
- wherein pyruvate-flavodoxin/ferredoxin oxidoreductase enzyme activity is increased within the genetically modified microorganism under aerobic or partially aerobic conditions during the stationary phase or non-dividing cellular state to produce an acetyl CoA pool; and
- wherein sugar uptake is enhanced within the genetically modified microorganism, when compared to a non-genetically modified microorganism.

20. The genetically modified microorganism of claim 19, wherein the increased pyruvate-flavodoxin/ferredoxin oxidoreductase enzyme activity is due to overexpression of a gene encoding pyruvate ferredoxin oxidoreductase during the stationary phase or non-dividing cellular state.

21. The genetically modified microorganism of claim 19, wherein the pyruvate-flavodoxin/ferredoxin oxidoreductase enzyme is encoded by the *ydbK* gene and the genetically modified microorganism is an *Enterobacter* microorganism.

22. The genetically modified microorganism of claim 19, wherein the increased pyruvate ferredoxin oxidoreductase enzyme activity is due to induction of oxidative soxRS regulon during the stationary phase or non-dividing cellular state.
23. The genetically modified microorganism of claim 19, wherein the increased pyruvate ferredoxin oxidoreductase enzyme activity is increased as the result of reduced NADPH levels within the genetically modified microorganism during the stationary phase or non-dividing cellular state.
24. The genetically modified microorganism of claim 19, wherein the activity of at least one sugar transporter is increased to enhance sugar uptake.
25. The genetically modified microorganism of claim 24, wherein constitutive expression of a sugar transporter gene results in increased sugar transporter activity within the genetically modified microorganism.
26. The genetically modified microorganism of claim 24, wherein the sugar transporter is conditionally overexpressed during the stationary phase or non-dividing cellular state.
27. The genetically modified microorganism of claim 24, wherein the sugar transporter is encoded by a *pts* gene.
28. The genetically modified microorganism of claim 19, wherein the microorganism is an *Enterobacter* microorganism.
29. The genetically modified microorganism of claim 19, wherein the microorganism is an *E. coli* microorganism.

30. The genetically modified microorganism of claim 19, wherein the enzyme of the production pathway is citramalate synthase.

31. The genetically modified microorganism of claim 19, wherein the product is pyruvate.

32. The genetically modified microorganism of claim 19, wherein the synthetic metabolic valves effect gene silencing by CRISPR interference, synthetic metabolic valves further comprising 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, the guide array comprising more than one promotor for each gene.

33. A bioprocess for production of a protein product from the genetically modified microorganism of claim 19, the bioprocess comprising:
in a first stage, growing the genetically modified microorganism in a medium and
in a second stage, upon depletion of a limiting nutrient from a growth medium, inducing a stationary phase or non-dividing cellular state,
wherein the genetically modified microorganism in the stationary phase or non-dividing cellular state produces product at a rate of 30 g/L or greater.

34. The bioprocess of claims 33, wherein the increased activity of a pyruvate-flavodoxin/ferredoxin oxidoreductase enzyme is caused by overexpression of a gene encoding an active pyruvate ferredoxin oxidoreductase, induction of oxidative soxRS regulon, reducing NADPH levels, reducing glucose-6-phosphate dehydrogenase levels with a synthetic metabolic valve directed to gene silencing of the *zwf* gene or selective proteolysis of the glucose-6-phosphate dehydrogenase enzyme, the valve activated in the stationary phase or non-dividing cellular state, or a combination thereof.

35. The bioprocess of claim 33, wherein the activity of at least one sugar transporter is increased.

36. The bioprocess of claim 33, wherein the product is citramalate, an enzyme of the production pathway comprises citramalate synthase, and the bioprocess produces citramalate at or greater than 100 g/L.

37. The bioprocess of claim 36, wherein the citramalate synthase enzyme is encoded by the cimA3.7 gene.

38. The bioprocess of claim 33, wherein the genetically modified microorganism comprises a plasmid comprising a citramalate synthase gene is operably linked to a low phosphate inducible promotor.