CLAIMS

1. A multi-stage fermentation bioprocess for producing a product from a genetically modified microorganism comprising:

providing a genetically modified microorganism for producing a product;

growing the provided genetically modified microorganism in a media in a growth phase, the genetically modified microorganism comprising:

i. a production pathway comprising at least one production enzyme for biosynthesis of the product; and

ii. one or more synthetic metabolic valves for reducing or eliminating flux through multiple metabolic pathways within the GMO when the synthetic metabolic valves are induced, the one or more synthetic metabolic valves comprising:

a) at least one silencing SMV that silences gene expression of a gene encoding an enzyme, or

b) at least one proteolytic SMV that controls proteolysis of an enzyme;

transitioning to a productive stationary phase wherein, compared to a microorganism lacking the production pathway or the one or more synthetic metabolic valves, the growth of the genetically modified microorganism is slowed or stopped and product production is enhanced, the productive stationary phase being entered by at least inducing the one or more synthetic metabolic valves; and

producing the product.

2. The multi-stage fermentation bioprocess of claim 1, wherein the product is selected from the group consisting of an amino acid, acetate, acetoin, acetone, acrylic, malate, fatty acid ethyl esters, isoprenoids, glycerol, ethylene glycol, ethylene, propylene, butylene, isobutylene, ethyl acetate, vinyl acetate, 1,4-butanediol, 2,3-butanediol, butanol, isobutanol, sec-butanol, butyrate, isobutyrate, 2-OH-isobutryate, 3-OH-butyrate, ethanol, isopropanol, D-lactate, L-lactate, pyruvate, itaconate, levulinate, glucarate, glutarate, caprolactam, adipic acid, propanol, isopropanol, fused alcohols, and 1,2-propanediol, 1,3-propanediol, formate, fumaric acid, propionic acid, succinic acid, valeric acid, maleic acid and poly-hydroxybutyrate.

3. The multi-stage fermentation bioprocess of claim 1, wherein:

the enzyme that is silenced by the silencing metabolic valve and the enzyme subject to proteolysis by the proteolytic synthetic metabolic valve are each encoded by a gene selected from the group consisting of: fabI, gltA, ldp, zwf, and udhA; or

the silencing synthetic metabolic valve is characterized by CRISPR interference with gene expression and expression of a CASCADE plasmid comprising an array of guide RNA genes; or

the proteolytic synthetic metabolic valve is characterized by expression of the proteolyzed enzyme operably linked to a N-terminal or C-terminal tag and controlled proteolysis is selective for enzymes comprising the N-terminal or C-terminal tag upon induction of the synthetic metabolic valve.

4. The multi-stage fermentation bioprocess of claim 1, wherein the step of providing a genetically modified microorganism for producing a product comprises:

providing a plurality of distinct genetically modified microorganisms, each genetically modified microorganism therein comprising:

i. a production pathway comprising at least one production enzyme for biosynthesis of a product; and

ii. a combination of one or more synthetic metabolic valves for reducing or eliminating flux through multiple metabolic pathways within the genetically modified microorganism when the synthetic metabolic valve is induced, the synthetic metabolic valve comprising:

a) a silencing SMV that silences gene expression of a gene encoding an enzyme, or

b) a proteolytic SMV that controls proteolysis of an enzyme,

wherein each of the plurality of genetically modified microorganisms is distinct therein by one or more of the production enzyme, the enzyme to be silenced or the enzyme to be proteolyzed;

independently growing each of the plurality of genetically modified microorganisms in culture media in a growth phase;

transitioning to a productive stationary phase where growth of at least one of the plurality of genetically modified microorganisms is slowed or stopped and product production is enhanced, as compared to a microorganism lacking the production pathway or synthetic metabolic valve, the productive stationary phase being entered by at least inducing the synthetic metabolic valve;

producing the product;

measuring the level of product produced in the production phase for each of the plurality of genetically modified microorganisms after a period of time, and

selecting a genetically modified microorganism to be provided for a fermentation process based on the level of product produced.

5. The multi-stage fermentation bioprocess of claim 1, wherein:

the product is alanine;

the production enzyme or a promotor sequence of a gene operably linked to a gene encoding the production enzyme is heterologous to the species from which the genetically modified microorganism is derived; and

a heterologous enzyme of alanine production is one of: NADPH-dependent alanine dehydrogenase (ald), alanine exporter (alaE), glyceraldehyde-3-phosphate dehydrogenase (gapN) or a combination thereof.

6. The multi-stage fermentation bioprocess of claim 1, wherein:

the product is mevalonate or 3-HP;

the production enzyme or a promotor sequence operably linked to a gene encoding the production enzyme is heterologous to the species from which the genetically modified microorganism is derived; and

a heterologous enzyme of mevalonate or 3-HP production is one of: an NADPH dependent 3-hydroxypropionic dehydrogenase, an acetyl-CoA acetyltransferase, NADPH dependent HMG-CoA reductase, HMG-CoA synthase or a combination thereof.

7. A genetically modified microorganism strain comprising:

i. a production pathway comprising at least one production enzyme for biosynthesis of a product; and

ii. a combination of one or more synthetic metabolic valves capable of reducing or eliminating flux through multiple metabolic pathways within the genetically modified microorganism when the one more synthetic metabolic valves are induced, the one or more synthetic metabolic valves comprising:

a) at least one silencing synthetic metabolic valve that silences gene expression of a gene encoding an enzyme, or

b) at least one proteolysis synthetic metabolic valve that controls proteolysis of an enzyme; and

wherein growth of the genetically modified microorganism is slowed or stopped and product production is enhanced by inducing the synthetic metabolic valve, and

wherein at least one enzyme that is silenced by the silencing metabolic valve and at least one enzyme that is controlled by the proteolytic metabolic valve are each encoded by a gene selected from the group consisting of: fabI, gltA, ldp, zwf, and udhA.

8. The genetically modified microorganism strain of claim 7, wherein the product is alanine;

the enzyme of the production pathway is one of: NADPH-dependent alanine dehydrogenase (ald), alanine exporter (alaE), glyceraldehyde-3-phosphate dehydrogenase (gapN) or a combination thereof; and

the synthetic metabolic valve comprises a combination of silencing of gene expression of fabI, gltA1 or gltA2 and controlled proteolysis of fabI or udhA.

9. The genetically modified microorganism of claim 7, wherein the product is mevalonate or 3-HP;

the enzyme of the production pathway is one of: an NADPH dependent 3-hydroxypropionic dehydrogenase, an acetyl-CoA acetyltransferase, NADPH dependent HMG-CoA reductase, HMG-CoA synthase or a combination thereof; and

the synthetic metabolic valve comprises a combination of silencing of gene expression of fabI, gltA1 or gltA2 and controlled proteolysis of fabI or udhA.

10. A plurality of about 50 to about 500 genetically modified microorganism strains of claim 7, wherein each of the plurality of genetically modified microorganisms is distinct therein by one or more of the production enzyme, the enzyme to be silenced or the enzyme to be proteolyzed.