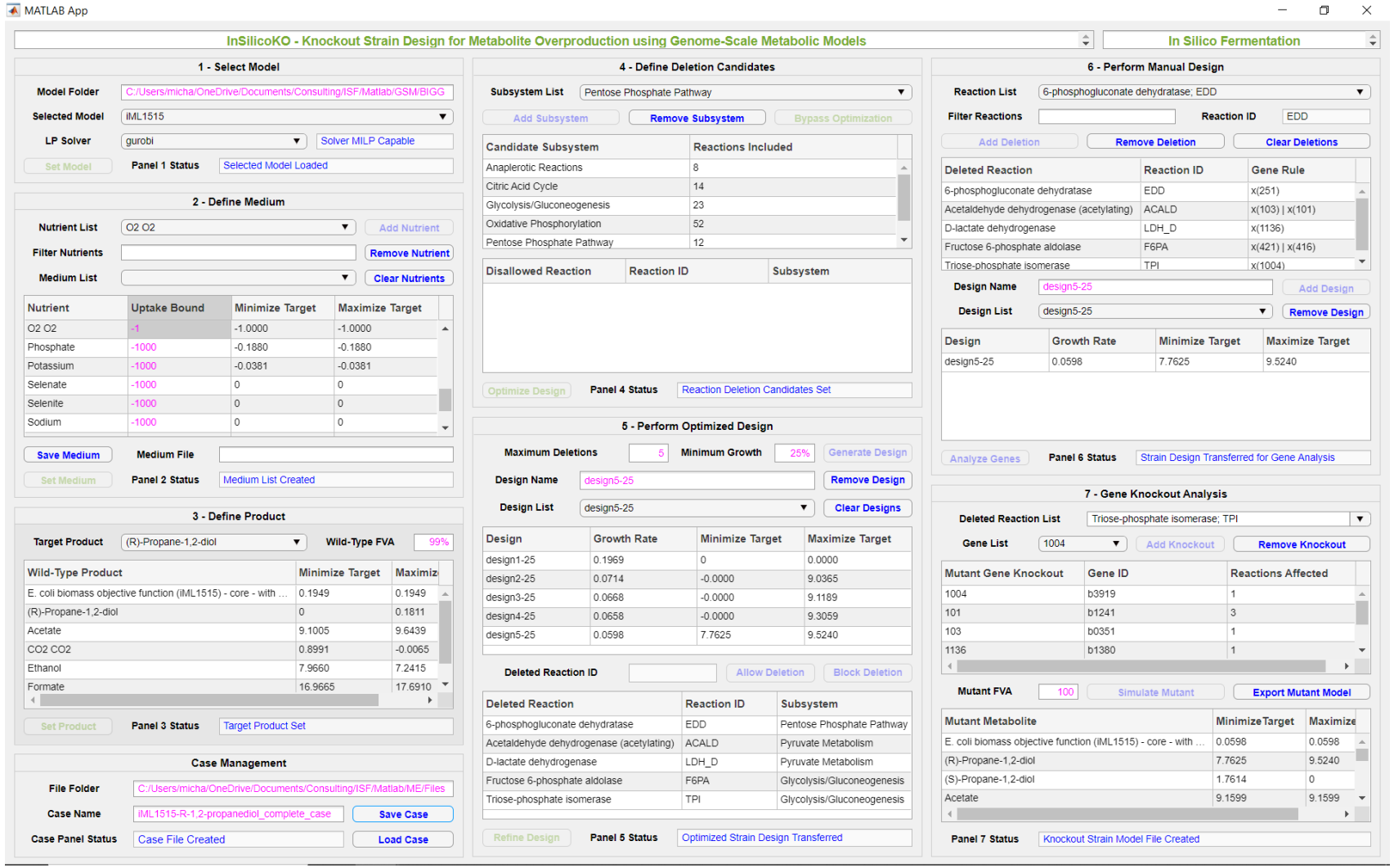
InSilicoKO – A MATLAB Application for Metabolite Overproduction Strain Design

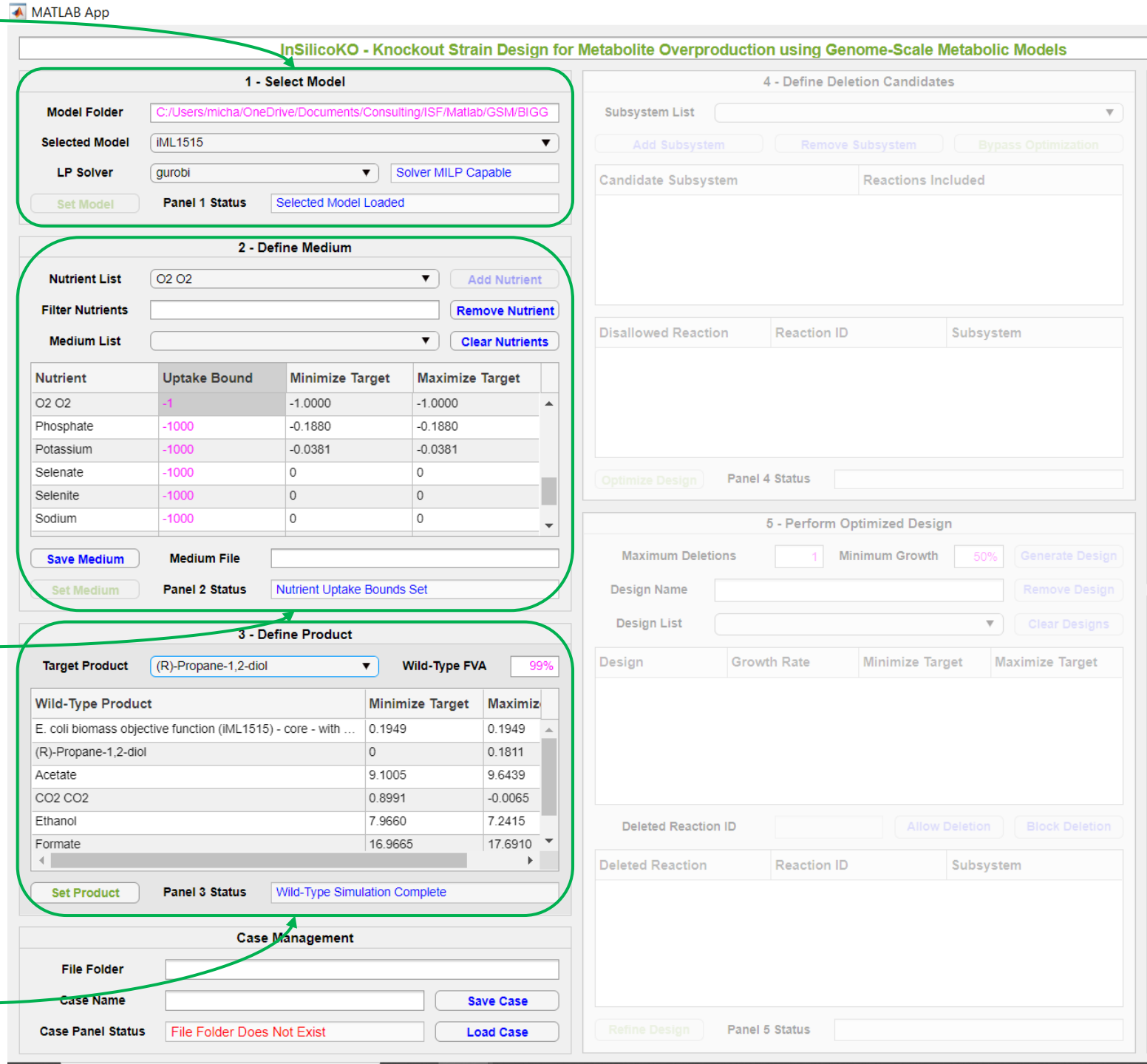


Example – Determine a set of gene knockouts to maximize the microaerobic synthesis of R-1,2-propanediol production in *Escherichia coli*

1. Select the metabolic model for the wild-type strain. Strain design optimization requires a LP solver with MILP capability.

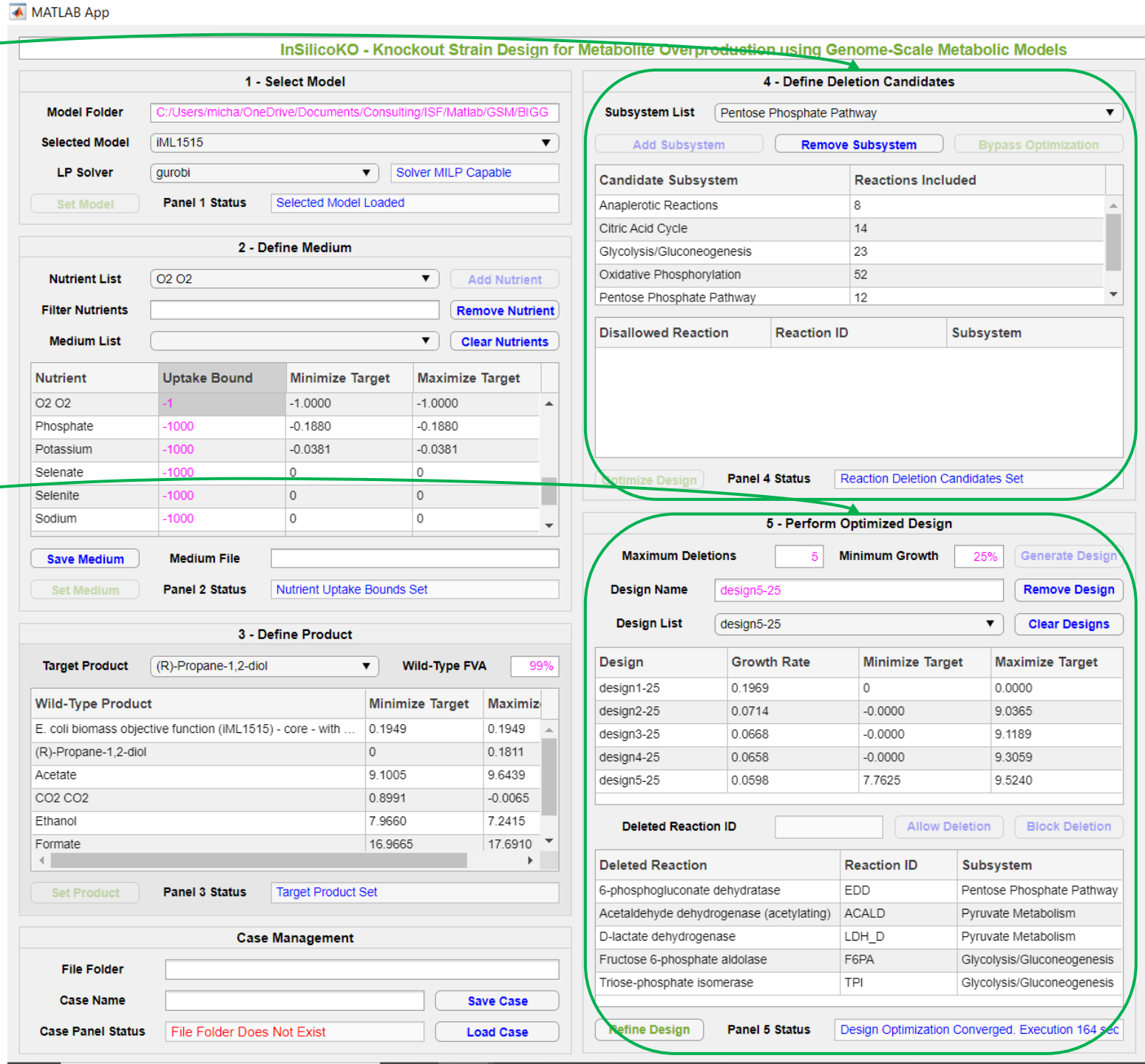
2. Define the culture medium in terms of supplied nutrients and their maximum uptake rates. The nutrients can be extracted from the model, specified manually, or loaded from a saved file.

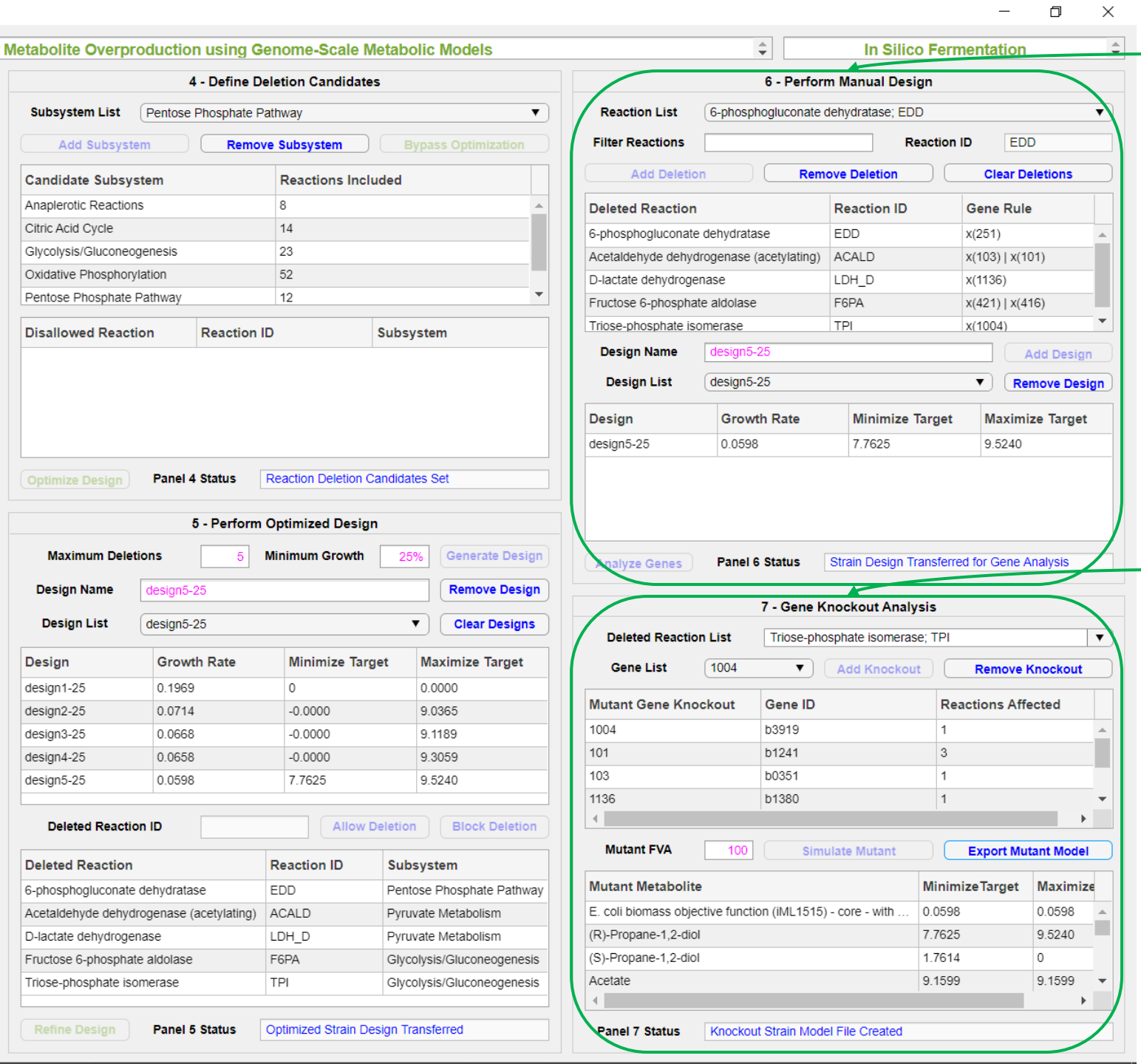
3. Define the secreted product to be maximized. FVA calculates ranges of nutrient uptake rates and product secretion rates of the wild-type strain.



4. Define the subsystems containing the desired reaction deletion candidates. Specific subsystem reactions selected by the optimizer can be excluded from subsequent designs.

5. Perform optimized strain design by specifying the number of deletions and allowable growth rate. FVA calculates the strain growth rate and product secretion range for the identified reaction deletions.

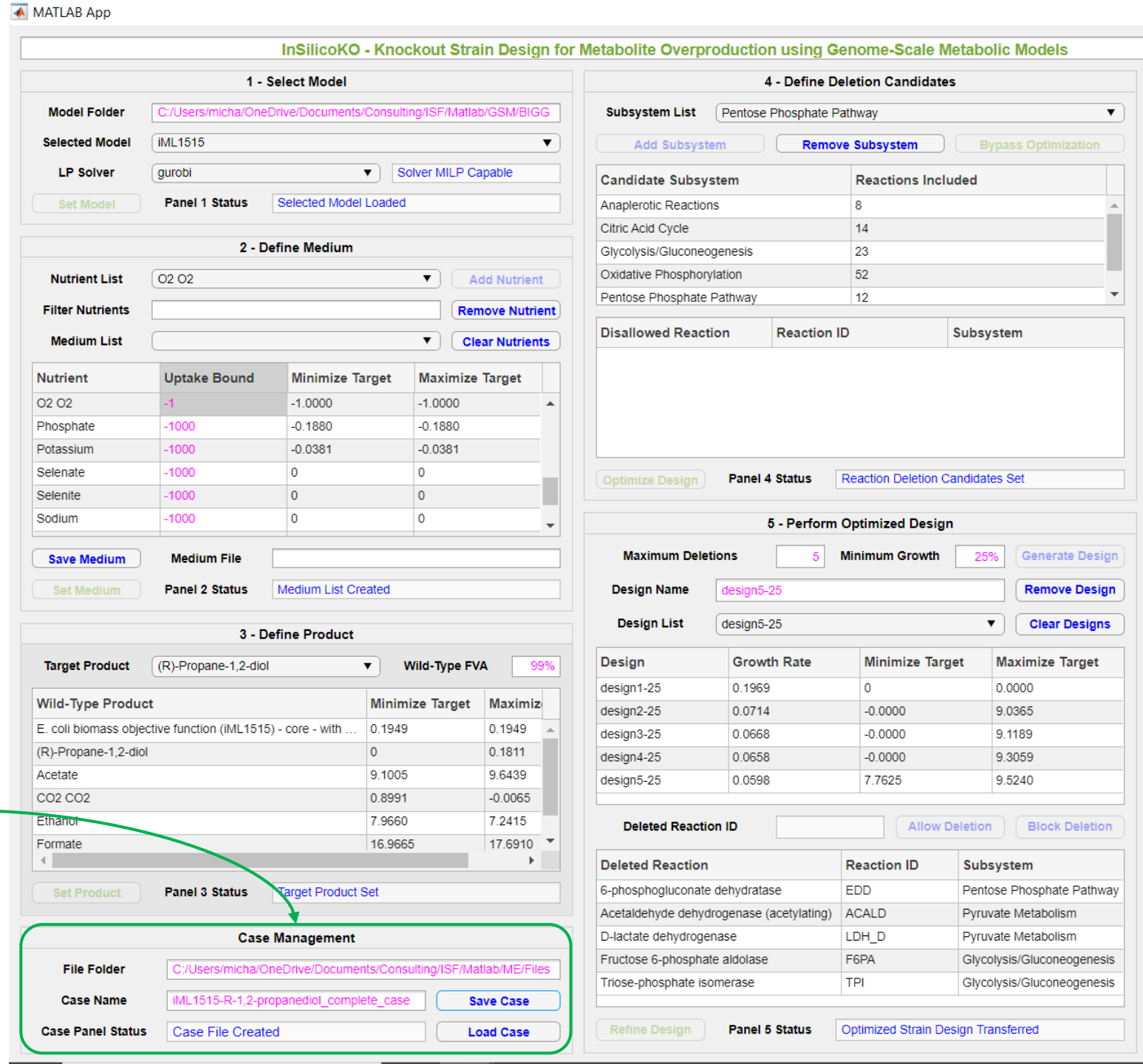




6. Modify the optimized designs or perform manual strain design in the absence of a MILP solver. Candidate reactions contained in the defined subsystems can be deleted or added.

7. Select gene knockouts to functionally eliminate the deleted reactions. FVA calculates ranges of nutrient uptake rates and product secretion rates of the knockout mutant strain.

The current state of the App can be saved and later reloaded to allow all work to be preserved. The gene knockout design can be saved as a COBRA metabolic model for use outside the App.



Gene Knockout Design for R-1,2-propanediol Overproduction in *Escherichia coli*

Wild-type Metabolic Model

- BIGG model iML1515
- Escherichia coli K-12 MG1655
- 2712 reactions, 1877 metabolites, 1516 genes

Specified Culture Medium

- Glucose minimal medium
- Microaerobic growth
- O2 uptake limited

Target Product

- R-1,2-propanediol
- Not secreted by wild-type strain at maximum growth rate

Candidate Reaction Deletions

- 6 subsystems
- 0 disallowed reactions
- 119 candidate deletions

Strain Design Optimization

- Maximum 5 reaction deletions
- Minimum 25% wild-type growth rate
- Best design: 5 deletions in glycolysis, pentose phosphate pathway & pyruvate metabolism

Optimized Design Refinement

- None

Gene Knockout Mutant

- 5 reaction deletions achieved with 7 gene knockouts
- Mutant growth rate 30% of wild-type growth rate
- R-1,2-propanediol secretion rate: 7.8-9.5 mmol/gDW/h
- Other major products are S-1,2-propanediol, acetate & CO_2