Robustness Analysis & Phenotype Phase Plane Analysis

Learning Objectives

- Explain the capabilities of robustness analysis
- Explain how shadow prices can be used in metabolic modeling
- Explain how reduced costs can be used in metabolic modeling
- Explain the capabilities of phenotype phase plane analysis

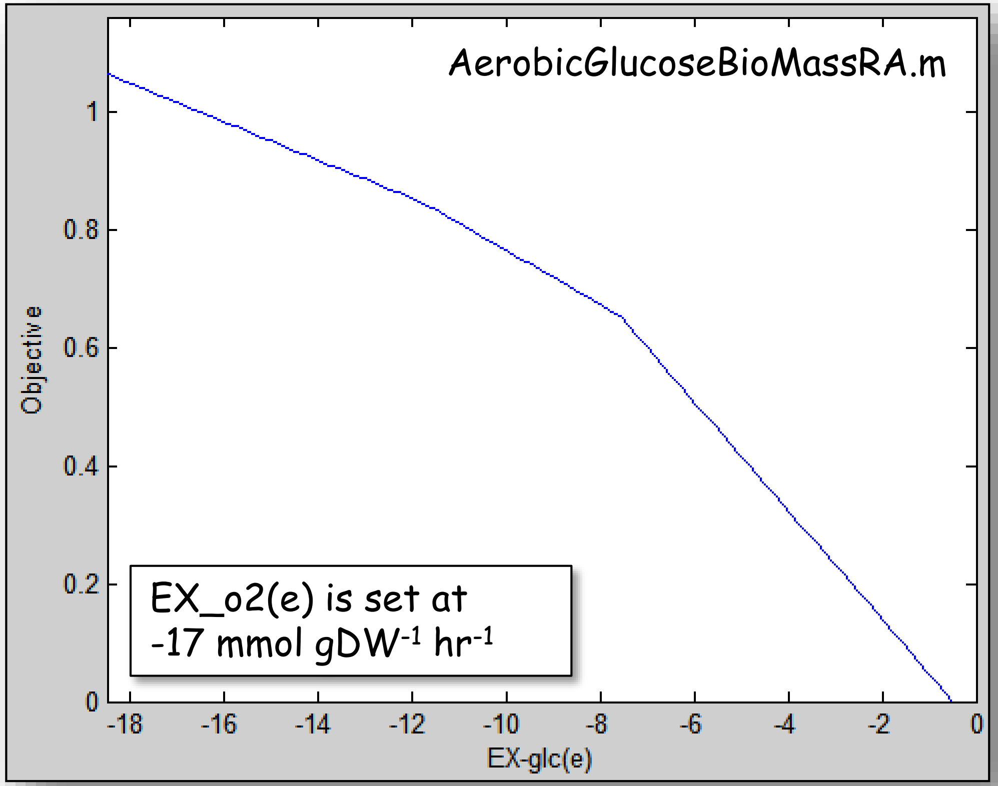
Lesson Outline

- Robustness Analysis
- Shadow Prices
- Reduced Costs
- Phenotype Phase Plane Analysis

Robustness Analysis

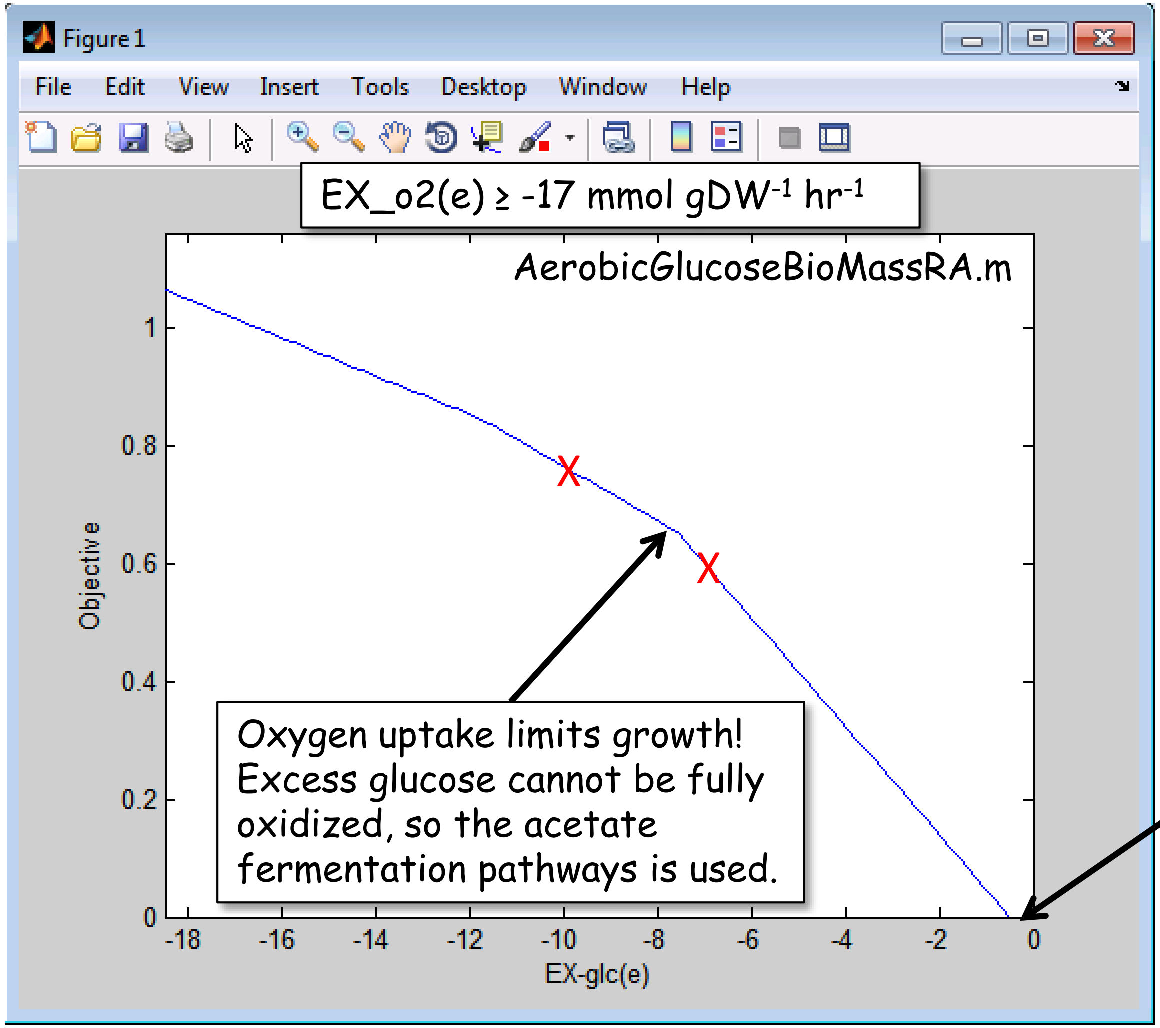
- The flux through one reaction is varied and the optimal objective value is calculated as a function of this flux. This reveals how sensitive the objective is to a particular reaction.
- Example: Determine the effect of varying glucose uptake on growth

```
Clear;

% Input model
model = readCbModel('ecoli_core_model.mat');
% Set oxygen uptake rate

model = changeRxnBounds(model,'EX_o2(e)',-17,'l');

% Set the upper bound for glucose uptake

model = changeRxnBounds(model,'EX_glc(e)',-18.5,'l');

% Set optimization objective

model = changeObjective(model,'Biomass_Ecoli_core_N(w/GAM)-Nmet2');

% Use robustnessAnalysis for glucose uptake rate

robustnessAnalysis(model,'EX_glc(e)',100);
```



Robustness Analysis Example

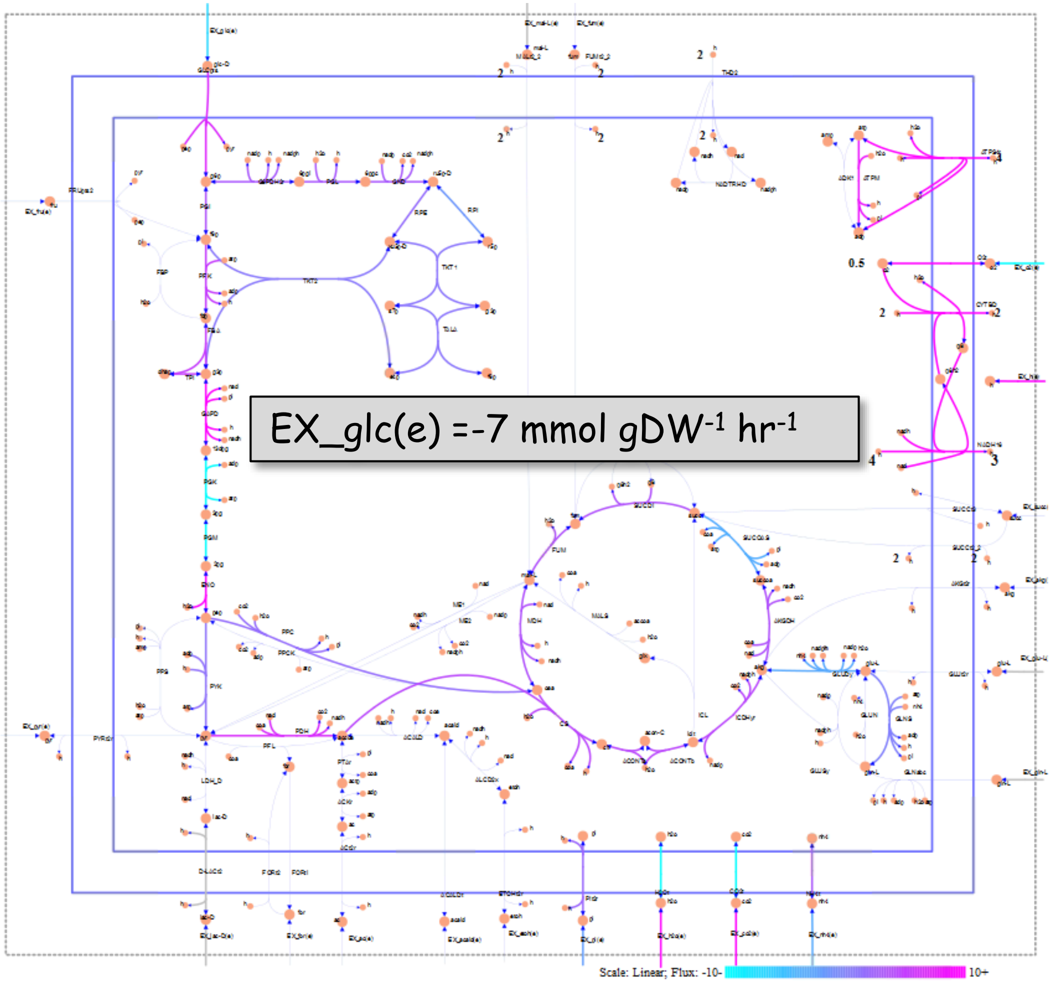
Impact of Increasing Glucose

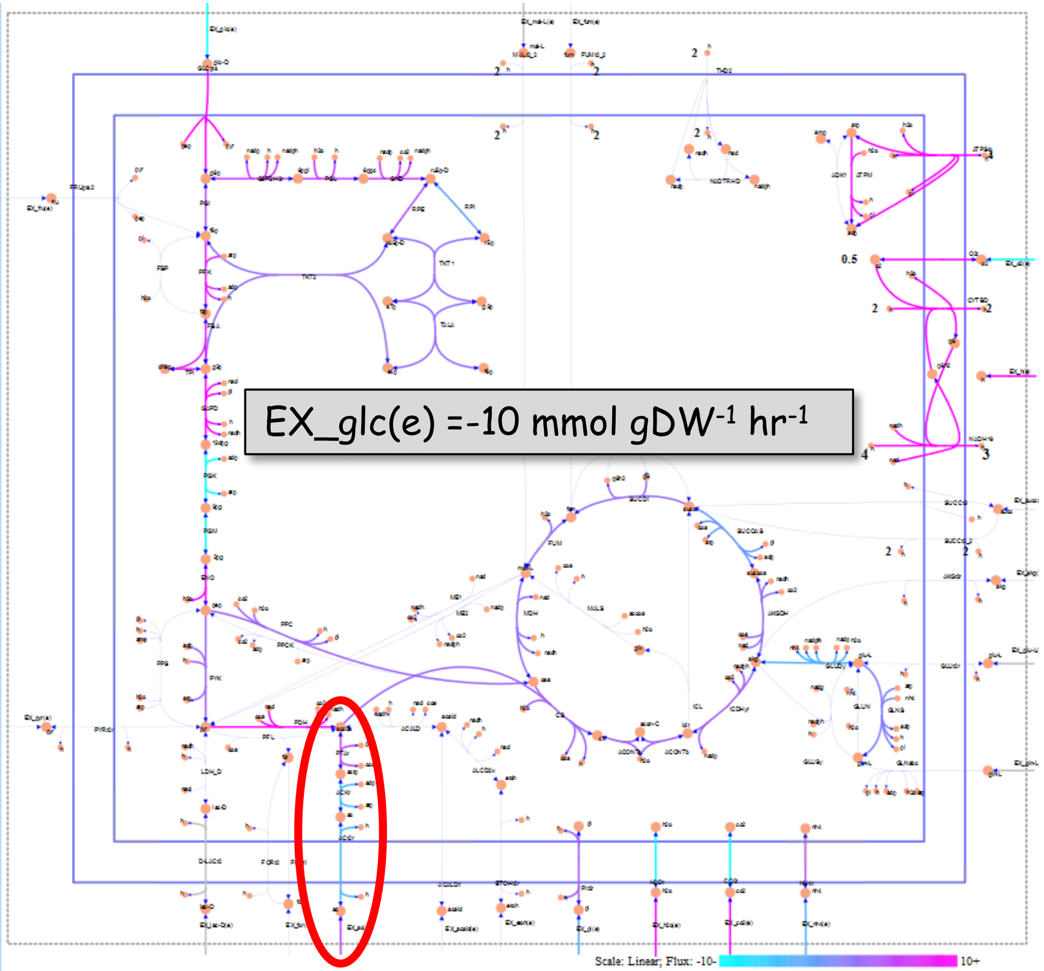


Growth remains at 0 hr^{-1} until a glucose uptake rate of about 0.48 mmol gDW^{-1} hr^{-1}, because with such a small amount of glucose, the system cannot make 8.39 mmol gDW^{-1} hr^{-1} of ATP needed to meet the default lower bound of the ATP maintenance reaction (ATPM)

Robustness Analysis Example Maps

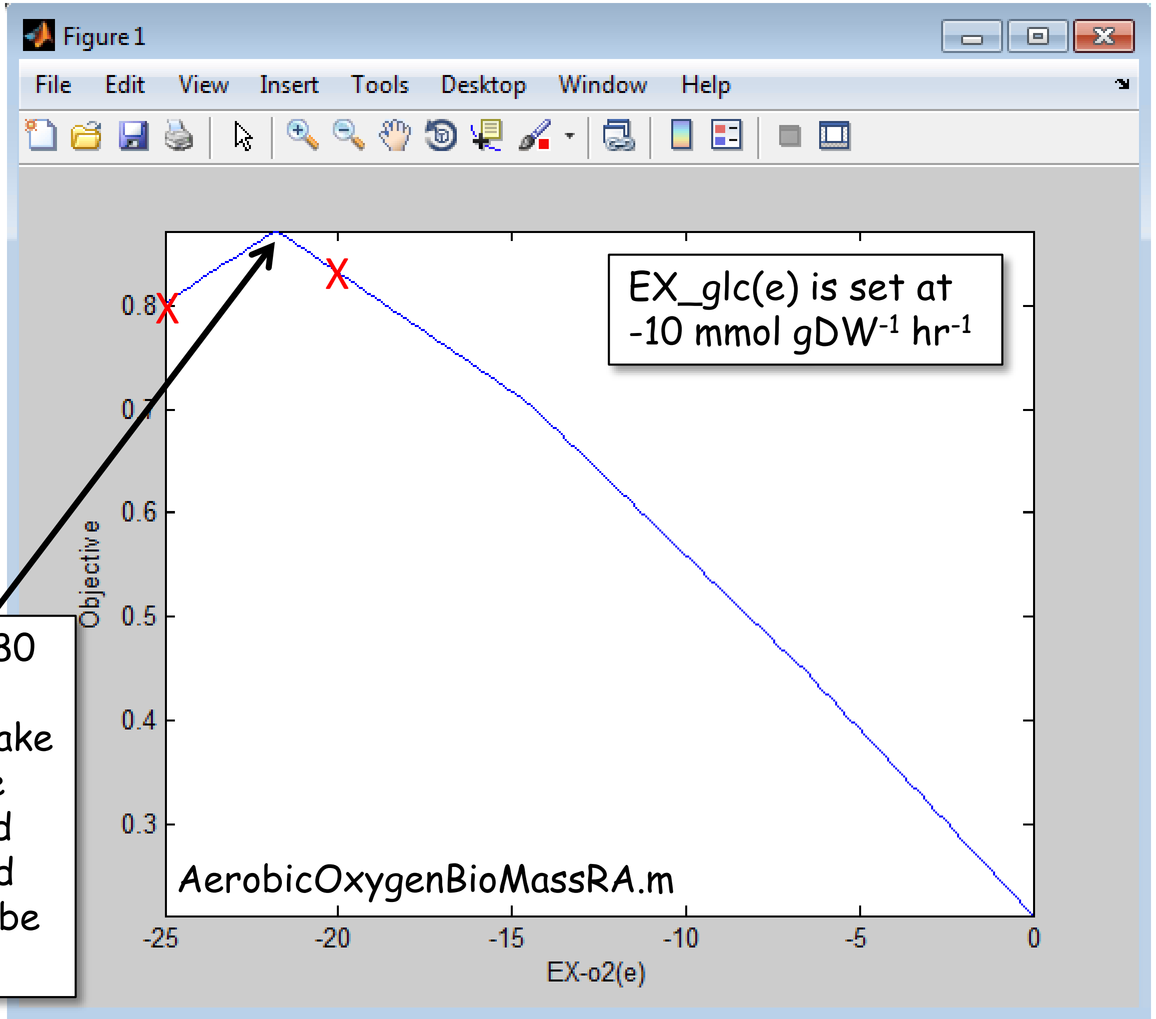
(AerobicGlucoseBioMassRA_Map.m)





Robustness Analysis Example

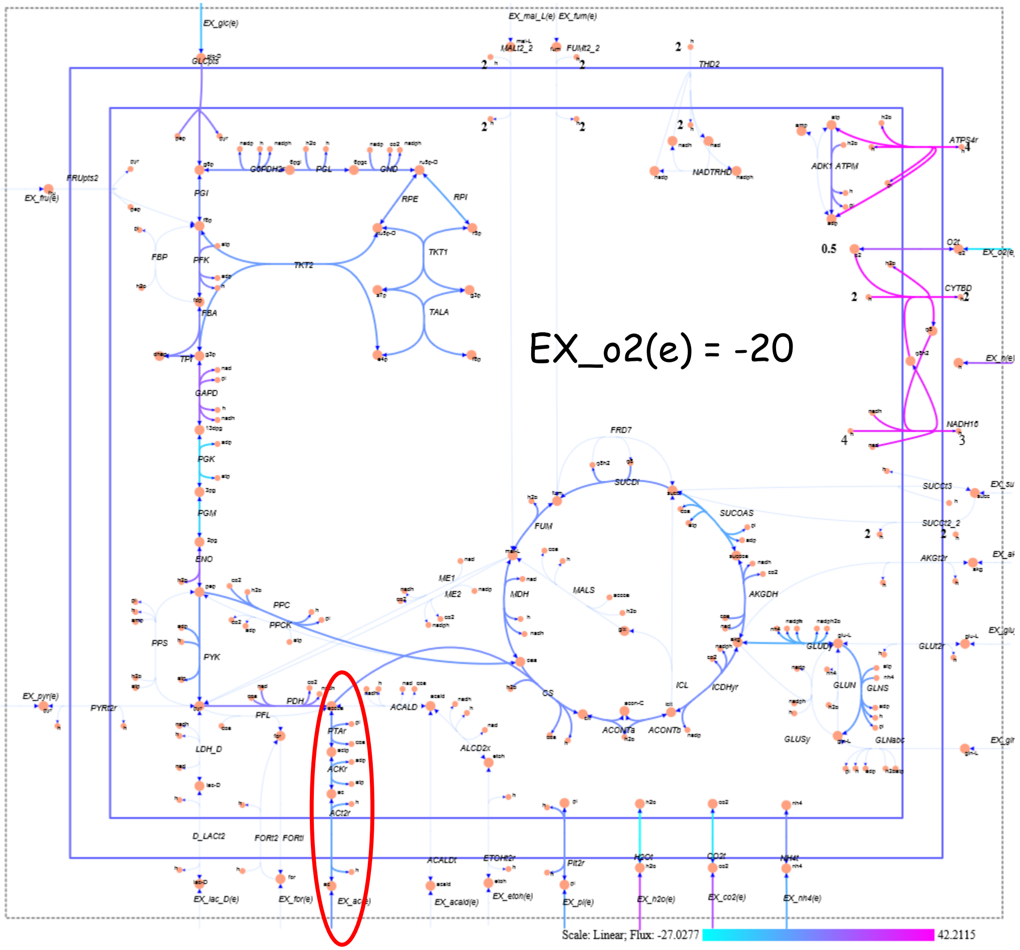
Impact of Increasing Oxygen

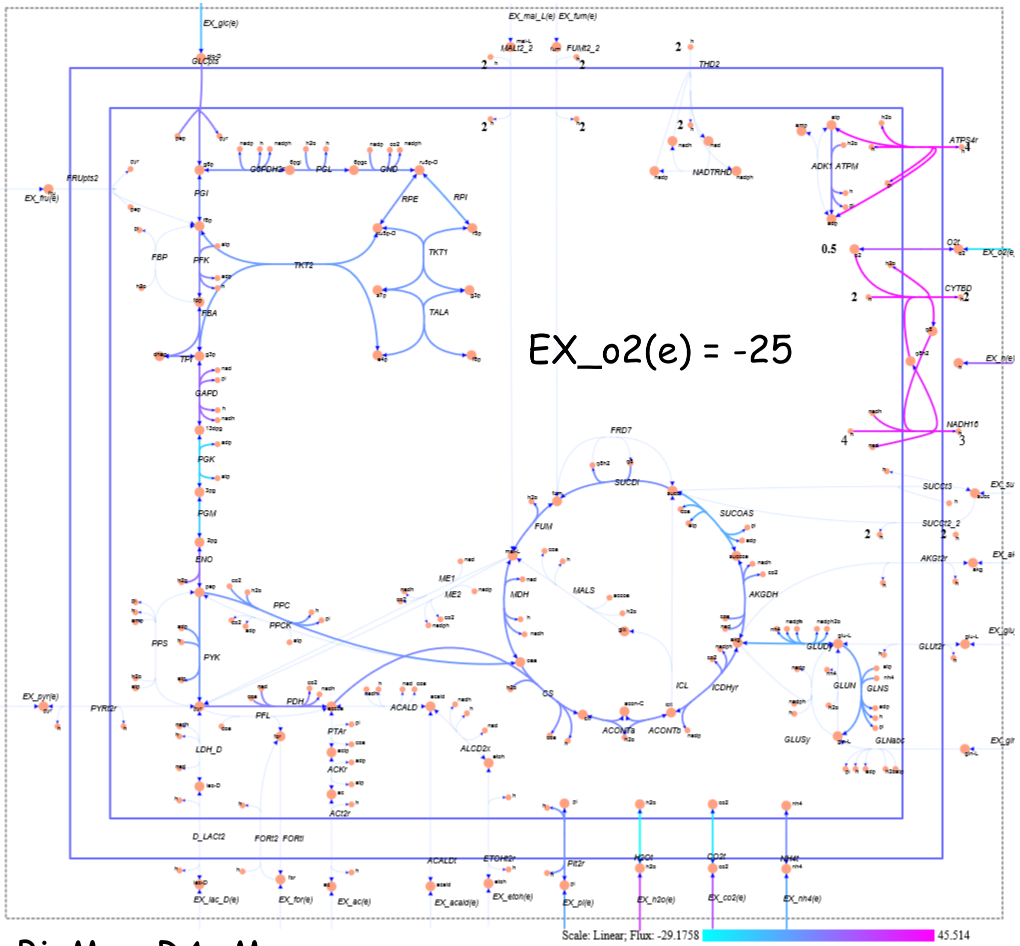


At an oxygen uptake of about 21.80 mmol gDW^{-1} hr^{-1}, growth actually begins to decrease as oxygen uptake increases. This is because glucose becomes limiting at this point, and glucose that would have been used to produce biomass must instead be used to reduce excess oxygen.

Robustness Analysis Example Maps

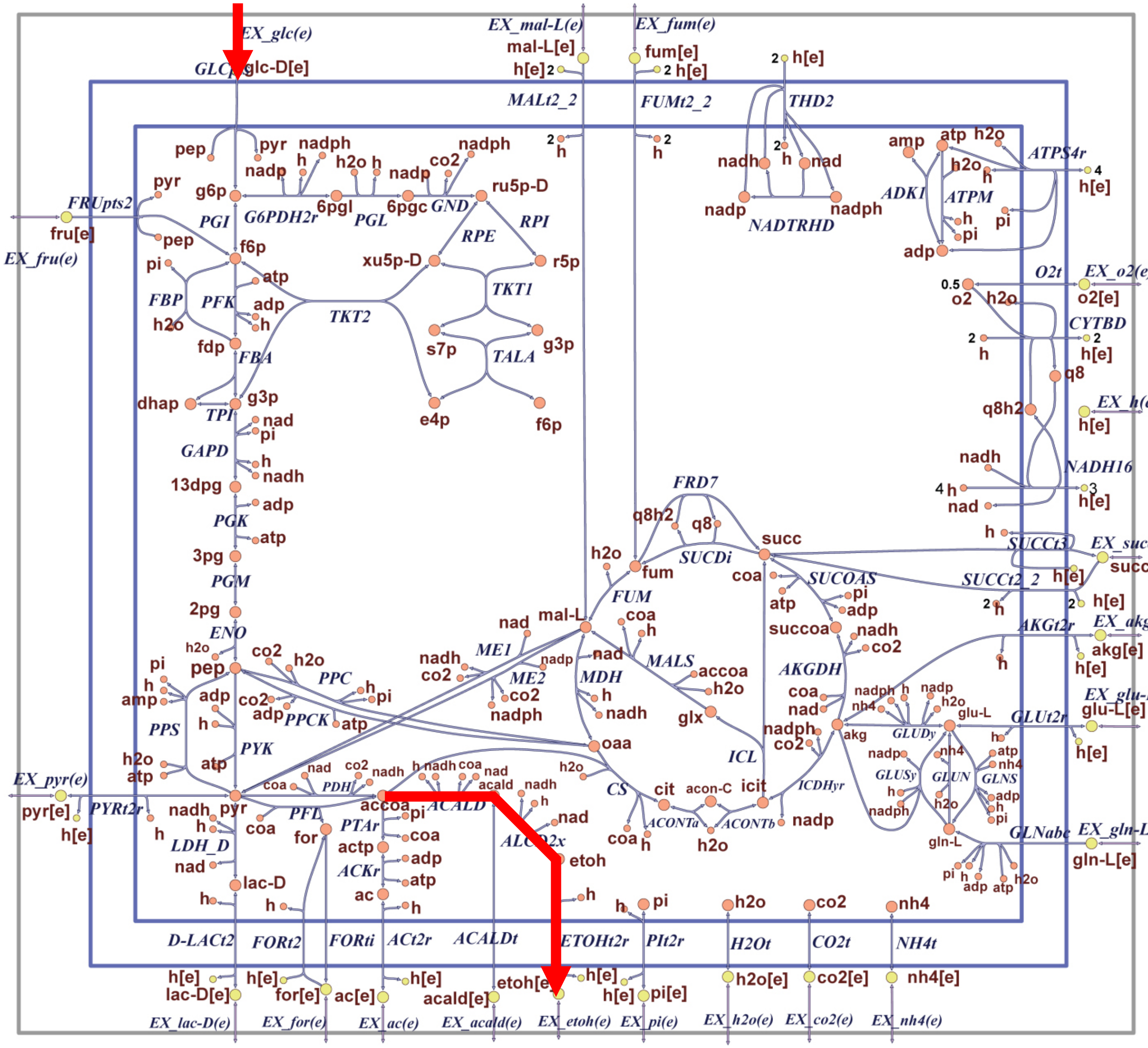
Impact of Increasing Oxygen





AerobicOxygenBioMassRA_Map.m

Anaerobic Ethanol Production



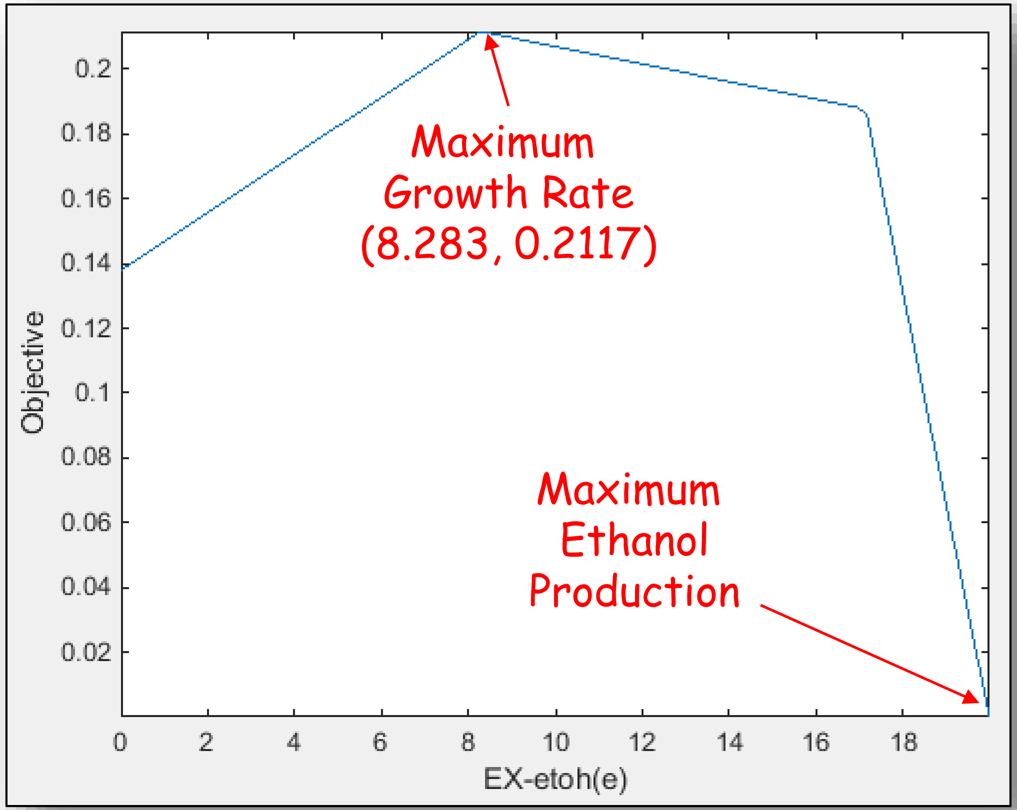
Orth, J. D., I. Thiele, et al. (2010). "What is flux balance analysis?" Nature biotechnology 28(3): 245-248.

Maximum Anaerobic Ethanol Production

AnaerobicEthanolRA.m

```
% AnaerobicEthanolRA.m
clear;

% Input the E.coli core model
model = readCbModel('ecoli_core_model.mat');

% Set uptake rates
model = changeRxnBounds(model,'EX_glc(e)',-10,'b');
model = changeRxnBounds(model,'EX_o2(e)',-0,'b');

% Set optimization objective to Biomass_Ecoli_core_N(w/GAM)-Nmet2
model = changeObjective(model,'Biomass_Ecoli_core_N(w/GAM)-Nmet2');

% Using robustnessAnalysis, plot the objective function as a function
% of the ethanol secretion rate

[controlFlux, objFlux] = robustnessAnalysis(model,'EX_etoh(e)',100);
```

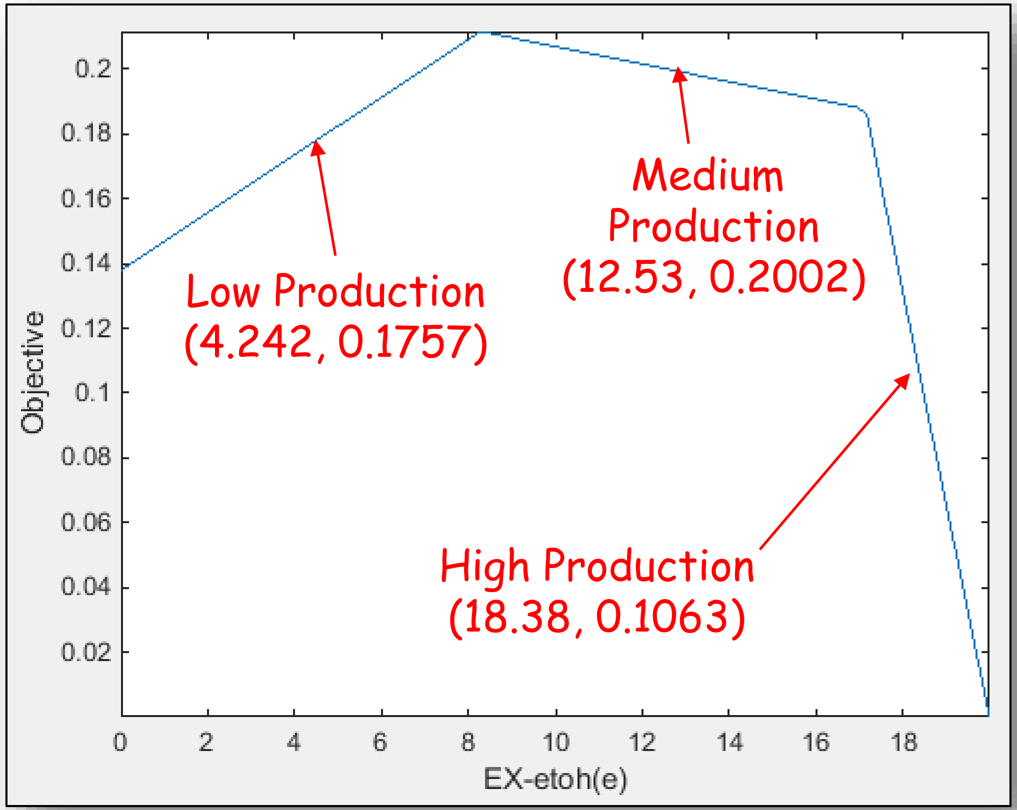
Fix Glucose and Oxygen Uptake Rates



Ethanol Production Phenotypes

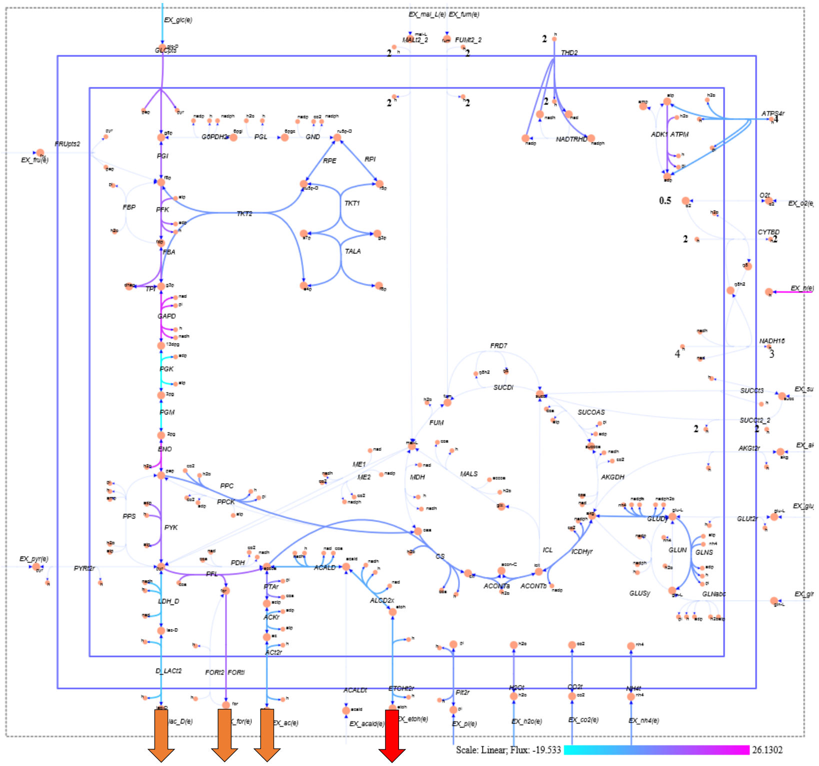
AnaerobicEthanolRA.m & AnaerobicEthanolRA_Maps.m

```
% Draw a map of the different production phenotypes
clear;
model = readCbModel('ecoli_core_model.mat');

model = changeRxnBounds(model,'EX_glc(e)',-10,'b');
model = changeRxnBounds(model,'EX_o2(e)',-0,'b');

model = changeRxnBounds(model,'EX_etoh(e)',4.242,'b'); % Low
%model = changeRxnBounds(model,'EX_etoh(e)',12.53,'b'); % Medium
%model = changeRxnBounds(model,'EX_etoh(e)',18.38,'b'); % High

model = changeObjective(model,'Biomass_Ecoli_core_N(w/GAM)_Nmet2');

FBAsolution = optimizeCbModel(model,'max',0,0)

map=readCbMap('ecoli_Textbook_ExportMap');
options.zeroFluxWidth = 0.1;
options.rxnDirMultiplier = 10;

drawFlux(map, model, FBAsolution.x, options);

printFluxVector(model, FBAsolution.x, true)
```

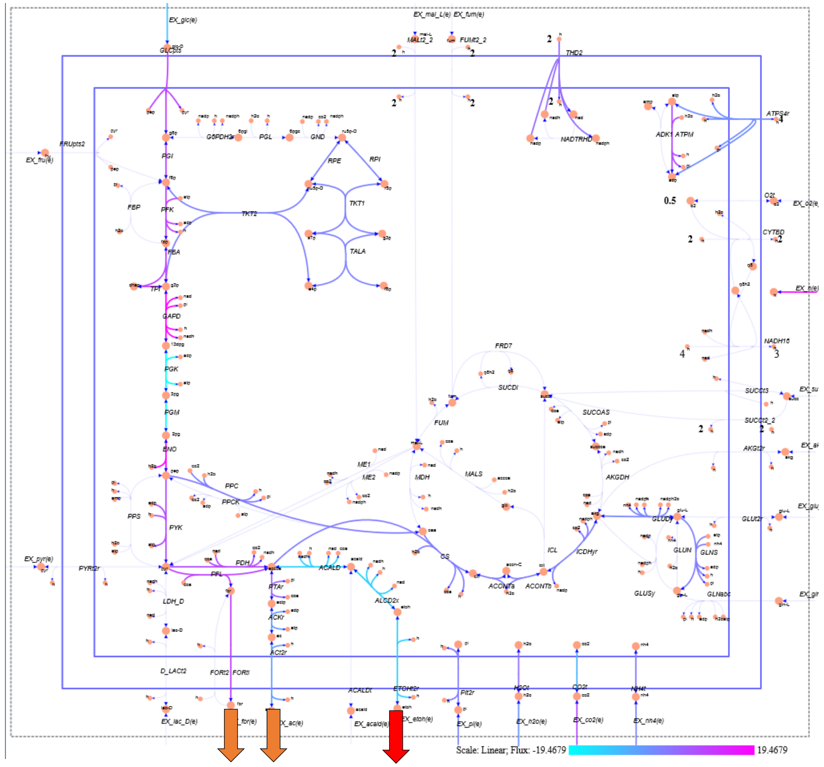


Ethanol Production Phenotype Maps

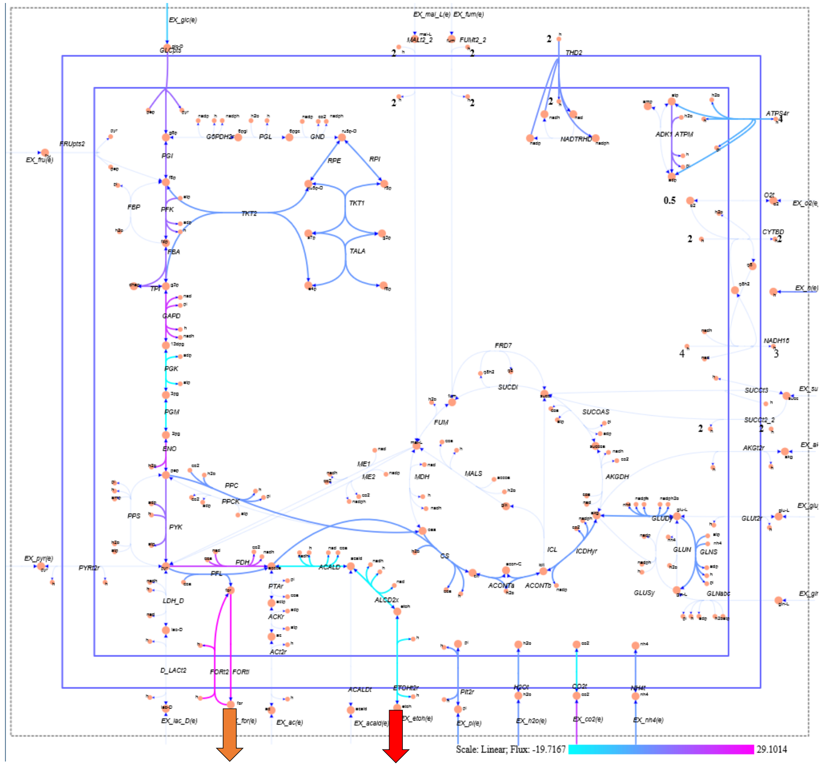
AnaerobicEthanolRA_Maps.m



Low Production



Medium Production



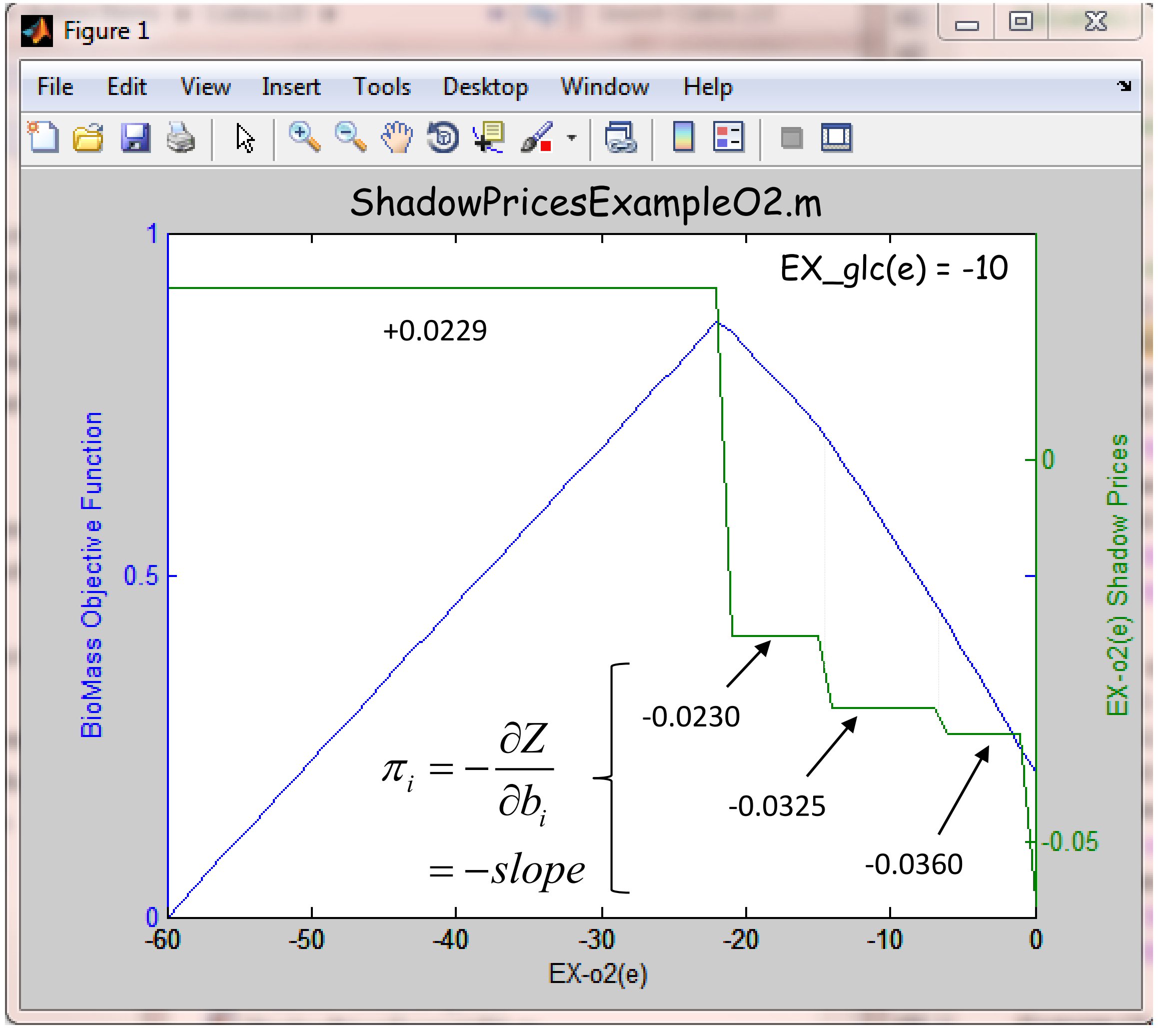
High Production

Lesson Outline

- Robustness Analysis
- Shadow Prices
- Reduced Costs
- Phenotype Phase Plane Analysis

Shadow Prices: Metabolites

- Shadow prices, π_i, are the derivative of the objective function, Z, with respect to the flux, b_i, of a metabolite.
- The shadow prices define the incremental change in the objective function if a constraining flux is incrementally changed.
- The sensitivity of an FBA solution is indicated by shadow prices. They indicate how much the addition of a given metabolite will increase or decrease the objective.
- In the COBRA Toolbox, shadow prices can be calculated by **optimizeCbModel**. The vector of y shadow prices is **solution.y**



Shadow Prices

Since

1 unit of flux of $b_{o2[e]}$

$$\pi_i = -\frac{\partial Z}{\partial b_i} \Rightarrow \pi_i \approx -\frac{\Delta Z_{GR}}{\Delta b_{o2[e]}} \Rightarrow \Delta Z_{GR} \approx -\pi_i \cancel{\Delta b_{o2[e]}}$$

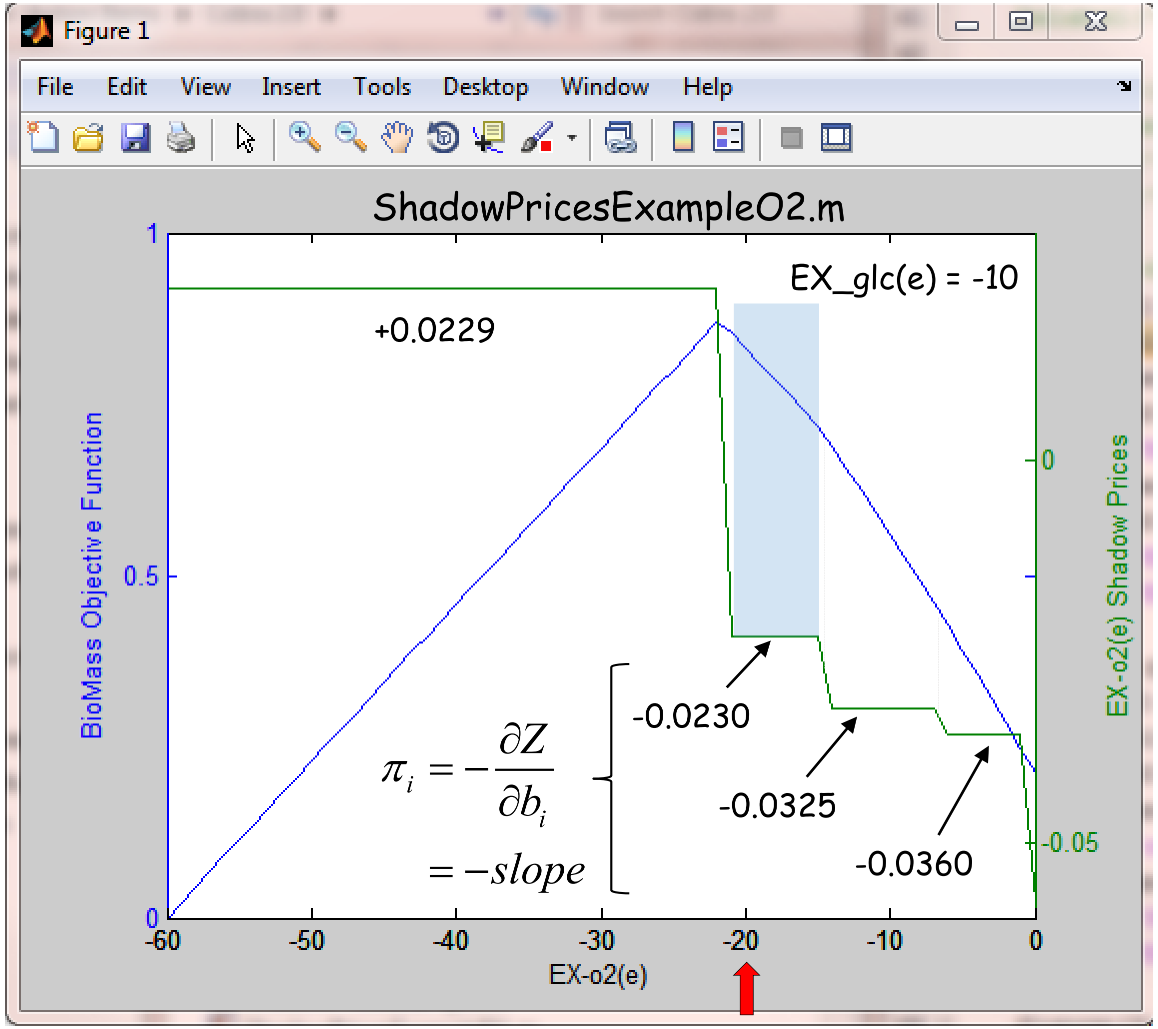
$$\Rightarrow \Delta Z_{GR} \approx -\pi_i$$

If the objective is set to maximize cell growth rate (Z_{GR}), and the shadow price (π_i), of oxygen ($b_{o2[e]}$) is -0.0230, it means that an additional flux unit of oxygen, o2[e], within the EX_o2(e) uptake region of -15 and -21, will increase the growth rate by 0.0230.

Each steady state solution (phenotype) will have different shadow prices. This example is based on EX_glc(e) = -10

Some solvers return positive shadow prices (gurobi, gplk)!

(AerobicGlucoseBioMassShadowPrices.m)



Shadow Prices for Growth on Glucose

(ShadowPricesAerobicGrowthRateData.m)

```
clear;
load('ecoli_core_model.mat');
% Set the lower bounds for oxygen and glucose uptake
model = changeRxnBounds(model,'EX_o2(e)',-20,'b ');
model = changeRxnBounds(model,'EX_glc(e)',-10,'l');
% Set optimization objective to Biomass_Ecoli_core_N(w/GAM)-Nmet2
model = changeObjective(model,'Biomass_Ecoli_core_N(w/GAM)-Nmet2');
% Optimize objective function
FBAsolution = optimizeCbModel(model,'max'); % Must allow loops
% Print flux values
printFluxVector(model, FBAsolution.x, true)
% Print shadow prices (Gurobi returns positive shadow prices )
disp('Shadow prices')
printShadowPriceVector(model, - FBAsolution.y, true) % In Matlab folder
```

Metabolite	SP
13dpg[c]	-0.0369835
2pg[c]	-0.0267812
3pg[c]	-0.0267812
6pgc[c]	-0.0573882
6pgl[c]	-0.0548376
ac[c]	-0.00255059
acald[c]	-0.0114776
acald[e]	-0.0114776
accoa[c]	-0.0102023
acon-C[c]	-0.0395341
actp[c]	-0.0127529
akg[c]	-0.0331576
akg[e]	-0.030607
amp[c]	0.0102023
atp[c]	-0.0102023
cit[c]	-0.0395341
dhap[c]	-0.0357082
e4p[c]	-0.04336
etoh[c]	-0.0127529
etoh[e]	-0.0102023
f6p[c]	-0.0586635
fdp[c]	-0.0714164
fru[e]	-0.0459106
fum[c]	-0.0280565
fum[e]	-0.0229553
g3p[c]	-0.0357082
g6p[c]	-0.0586635
glc-D[e]	-0.0459106

Metabolite	SP
gln-L[c]	-0.0471859
gln-L[e]	-0.0369835
glu-L[c]	-0.0369835
glu-L[e]	-0.0344329
glx[c]	-0.0178541
h[c]	0.00255059
icit[c]	-0.0395341
lac-D[c]	-0.0153035
lac-D[e]	-0.0127529
mal-L[c]	-0.0280565
mal-L[e]	-0.0229553
nad[c]	0.00382588
nadp[c]	0.00637647
o2[c]	-0.0229553
o2[e]	-0.0229553
oaa[c]	-0.0267812
pep[c]	-0.0267812
pi[c]	-0.00255059
pyr[c]	-0.0140282
pyr[e]	-0.0114776
q8h2[c]	0.00637647
r5p[c]	-0.0510117
ru5p-D[c]	-0.0510117
s7p[c]	-0.0663153
succ[c]	-0.02168
succ[e]	-0.0165788
succoa[c]	-0.0293318
xu5p-D[c]	-0.0510117

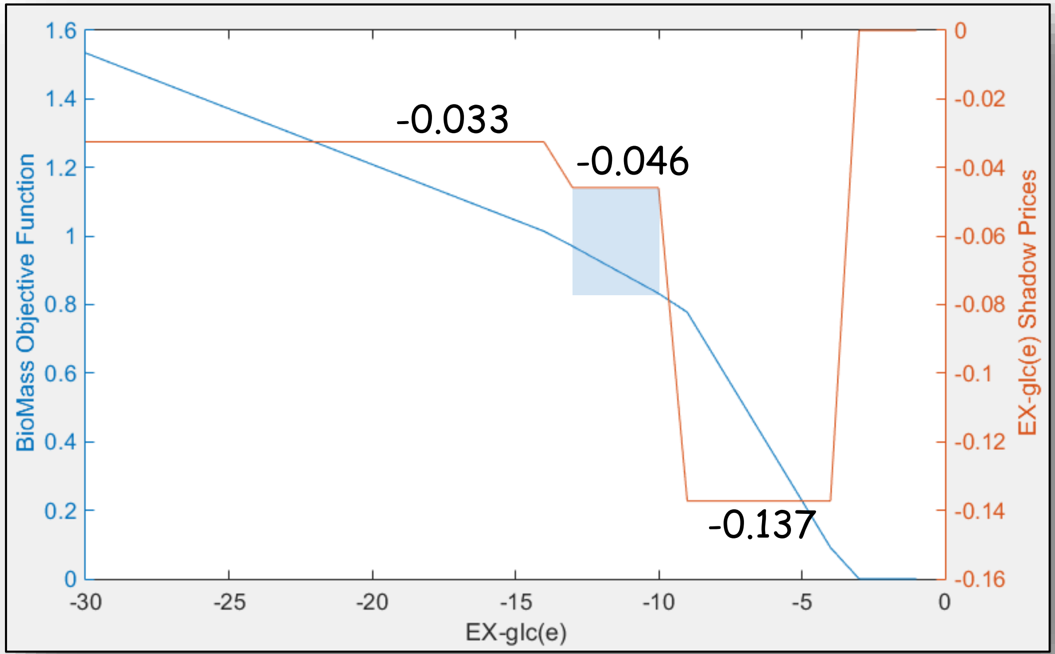
Glucose Shadow Prices: Aerobic Growth Rate Example

(ShadowPricesAerobicGrowthRate_glc.m)

If the objective is set to maximize cell growth rate (Z_{GR}), and the shadow price ($\pi_{glc\text{-}D[e]}$), of glucose is -0.046 when EX_o2(e) = -20, it means that an additional molecule of glucose will increase the growth rate by 0.046.

```
clear;
load('ecoli_textbook.mat');
model = changeRxnBounds(model,'EX_o2(e)',-20,'b ');
model = changeRxnBounds(model,'EX_glc(e)',-10,'b ');
model = changeObjective(model,'Biomass_Ecoli_core_N(w/GAM)_Nmet2');
FBAsolution = optimizeCbModel(model,'max')
printFluxVector(model, FBAsolution.x, true)
```

Change between -10 & -11



Biomass (EX_glc(e) = -10)	Biomass (EX_glc(e) = -11)	ΔBiomass
0.832	0.878	0.046

Lesson Outline

- Robustness Analysis
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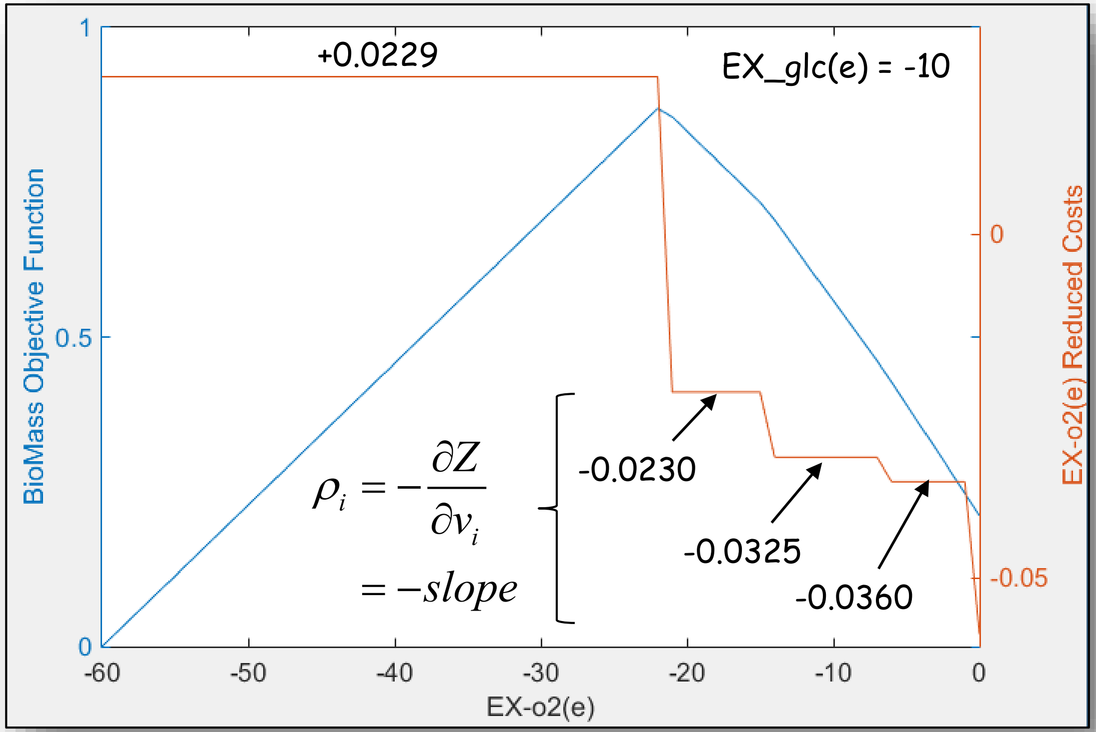
- Reduced Costs
- Phenotype Phase Plane Analysis

Reduced Costs: Reactions

- Reduced costs, ρ_i, are the derivatives of the objective function (Z) with respect to a reaction (v_i). Reduced costs indicate how much each particular reaction affects the objective.

1 unit of flux

$$\rho_{\mathrm{i}} = -\frac{\partial \mathrm{Z}}{\partial \mathrm{v}_{\mathrm{i}}} \Rightarrow \rho_{\mathrm{i}} \approx -\frac{\Delta \mathrm{Z}}{\Delta \mathrm{v}} \Rightarrow \Delta \mathrm{Z} \approx -\rho_{\mathrm{i}}\,\cancel{\Delta \mathrm{v}} \Rightarrow \Delta \mathrm{Z} \approx -\rho_{\mathrm{i}}$$



ReducedCostsExampleO2.m

- The reduced costs are associated with each flux (v_i) and signify the amount by which the objective function is decreased if v_i is increased. For instance, if the input flux of glucose shows a reduced cost of -x, it means that increasing that flux by one unit will increase of the objective function by x units.
- In the COBRA Toolbox, reduced costs can be calculated by optimizeCbModel. The vector of reduced costs is **FBAsolution.w**
- Some solvers return positive reduced costs (gurobi, gplk)

Glucose Reduced Costs: Aerobic Growth Rate Example

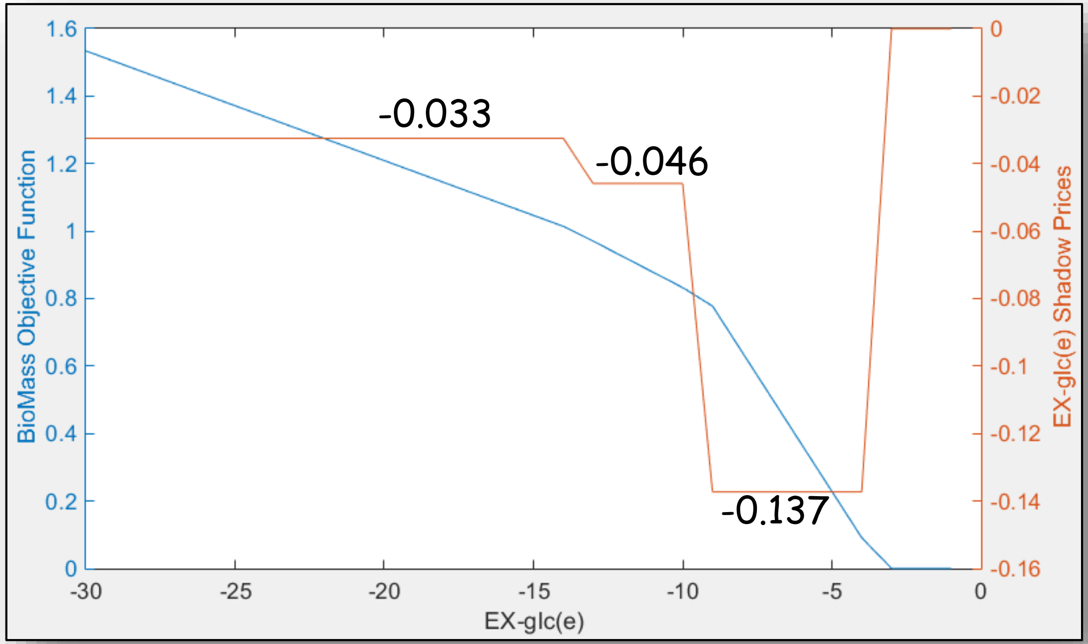
(ReducedCostsAerobicGrowthRate_glc.m)

If the objective is set to maximize cell growth rate (Z_{GR}), and the reduced costs ($\rho_{EX\_glc(e)}$), of glucose ($EX\_glc(e)$) is -0.046, it means that an additional unit of glucose will increase the growth rate by 0.046.

Change between -10 & -11

```
clear;
model=readCbModel('ecoli_textbook');
model = changeRxnBounds(model,'EX_o2(e)',-20,'b ');
model = changeRxnBounds(model,'EX_glc(e)',-10,'b ');
model = changeObjective(model,'Biomass_Ecoli_core_N(w/GAM)_Nmet2');
FBAsolution = optimizeCbModel(model,'max')
printFluxVector(model, - FBAsolution.x, true)
```

ReducedCostsExample_glc.m

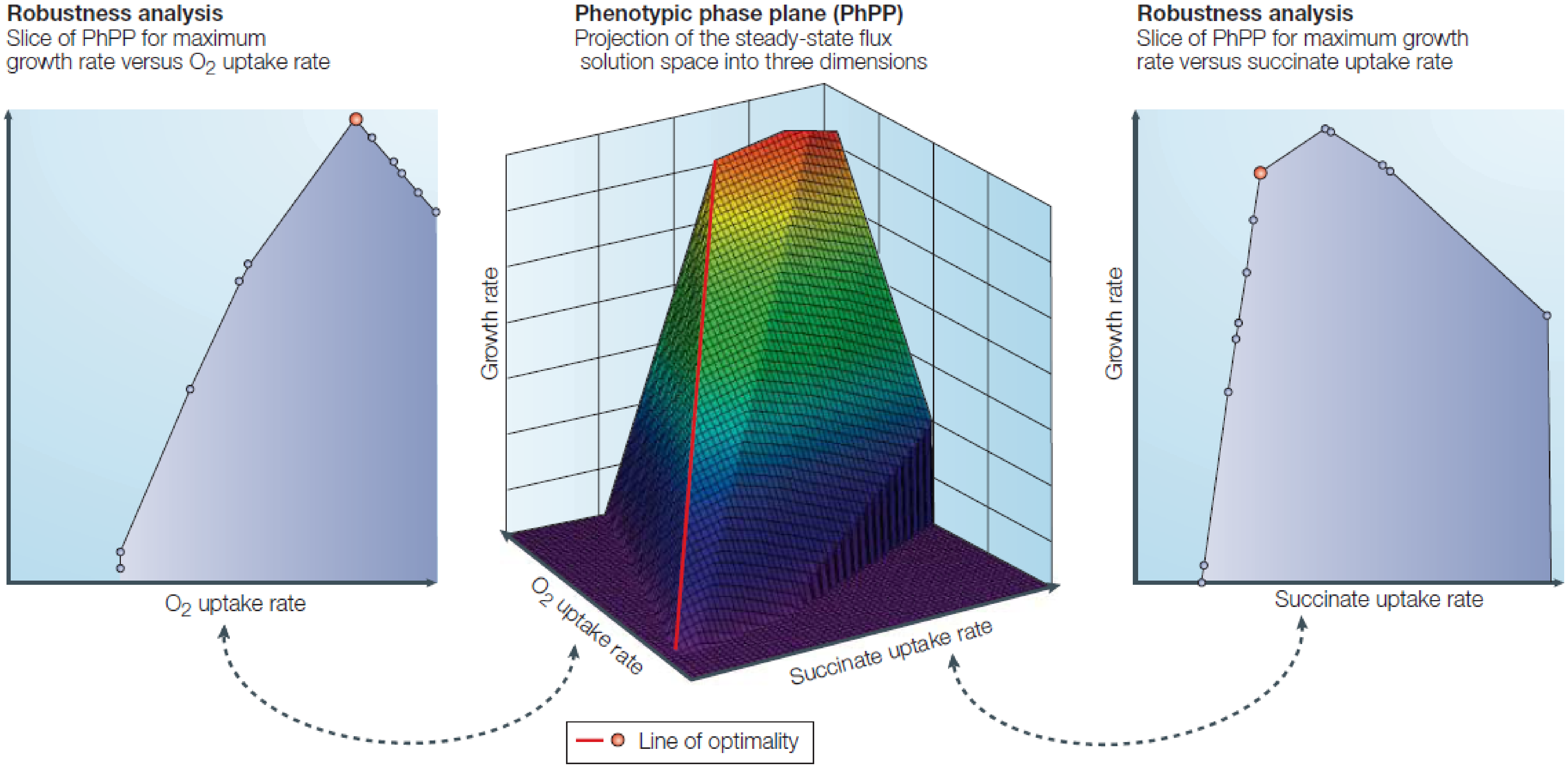


Biomass (EX_glc(e) = -10)	Biomass (EX_glc(e) = -11)	ΔBiomass
0. 832	0. 878	0.046

Lesson Outline

- Robustness Analysis
- Shadow Prices
- Reduced Costs
- Phenotype Phase Plane Analysis

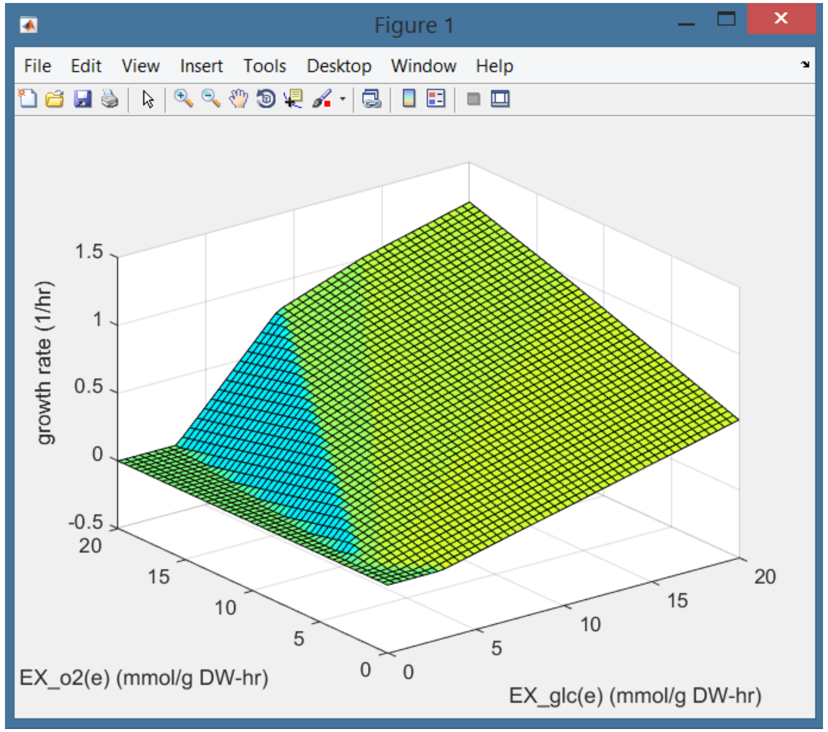
Phenotype Phase Plane



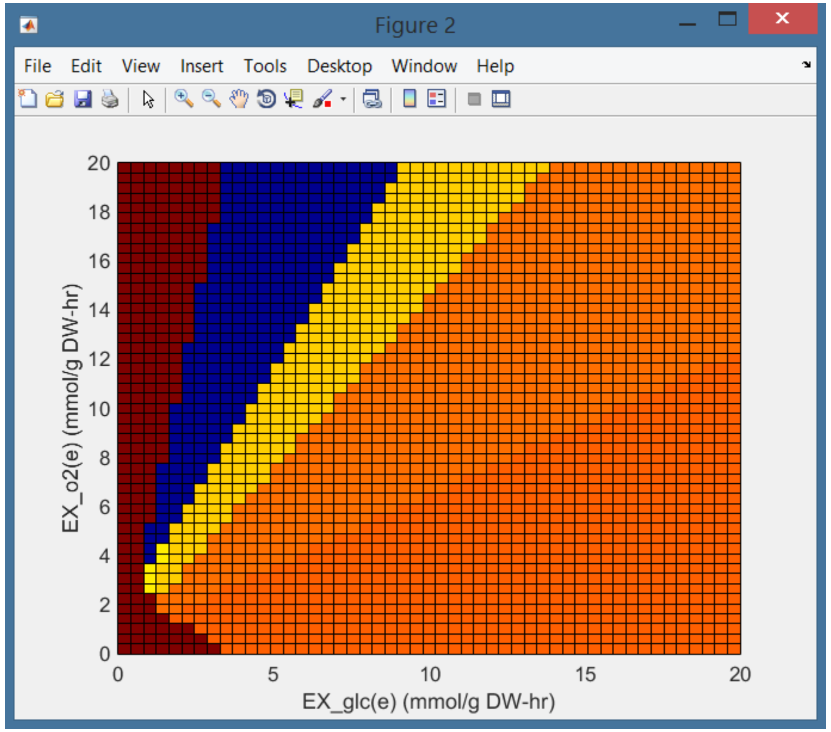
Price, N. D., J. L. Reed, et al. (2004). "Genome-scale models of microbial cells: evaluating the consequences of constraints." Nature reviews. Microbiology 2(11): 886-897.

Phenotype Phase Plane Analysis

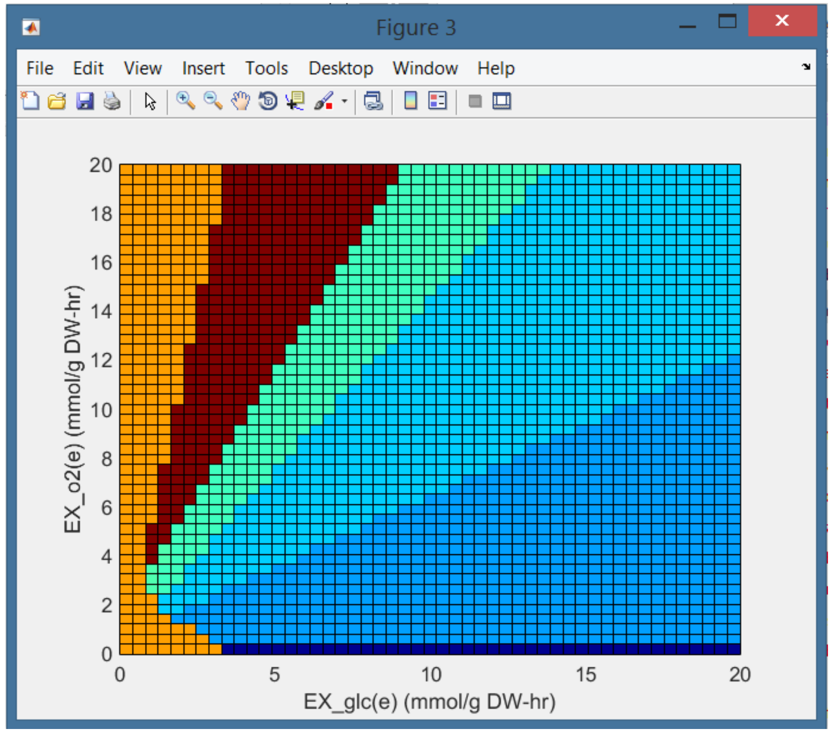
- It is also possible to vary two parameters simultaneously and plot the results as a phenotypic phase plane. These plots can reveal the interactions between two reactions.
- Matlab Cobra function: phenotypePhasePlane(model, ‘rxn1’,’rxn2’)



Phenotype Phase Plane



Shadow Prices of rxn1

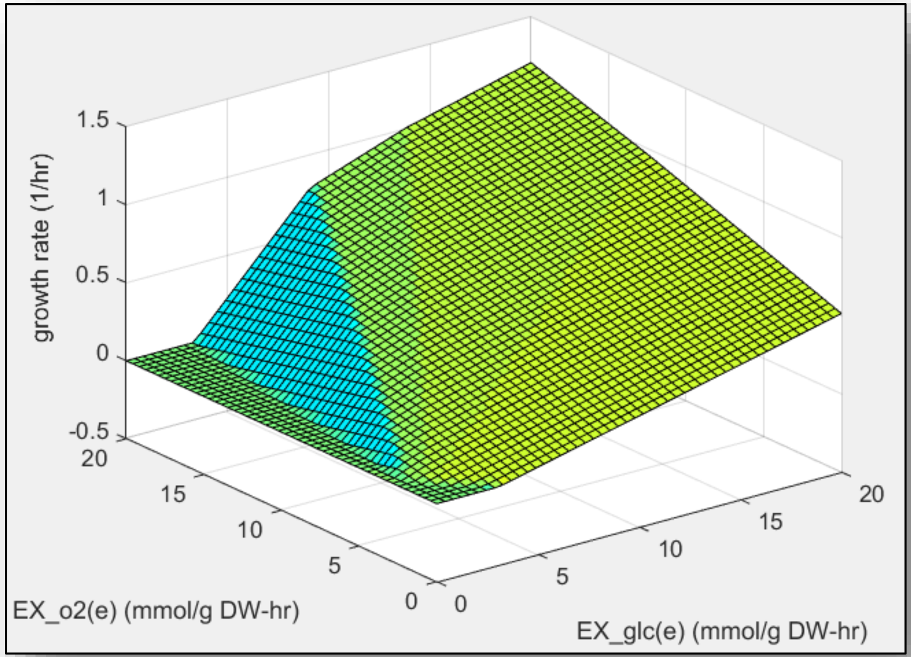


Shadow Prices of rxn2

Phenotype Phase Plane Analysis Example

AerobicGlucoseBioMassPPP.m

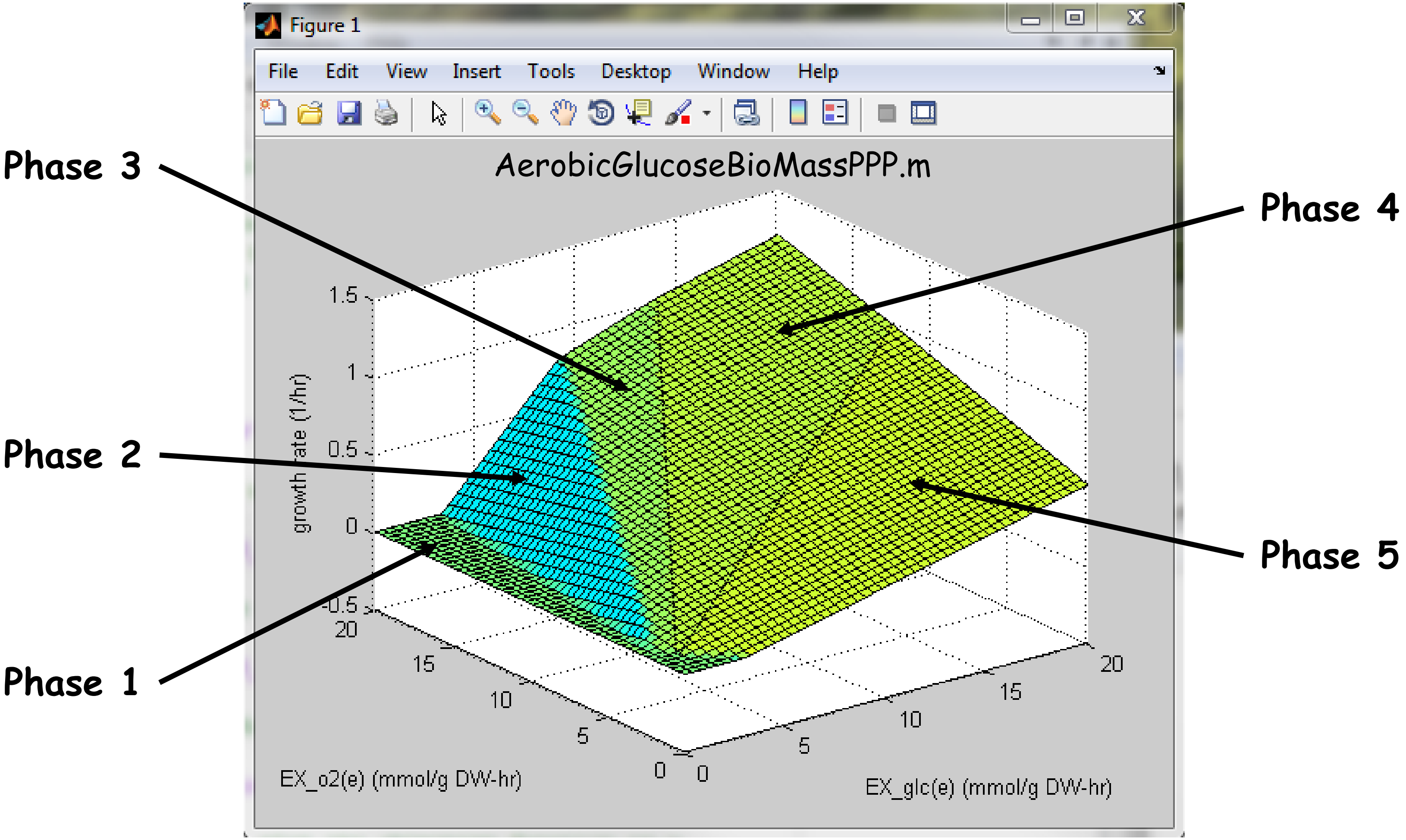
```
% Input model
model = readCbModel('ecoli_core_model.mat');
% Set oxygen and glucose uptake rates
model = changeRxnBounds(model,'EX_o2(e)',-20,'l');
model = changeRxnBounds(model,'EX_glc(e)',-20,'l');
% Set optimization objective
model = changeObjective(model,'Biomass_Ecoli_core_N(w/GAM)-Nmet2');
% Phenotype  phase plane analysis
phenotypePhasePlane(model,'EX_glc(e)', 'EX_o2(e)‘);
```



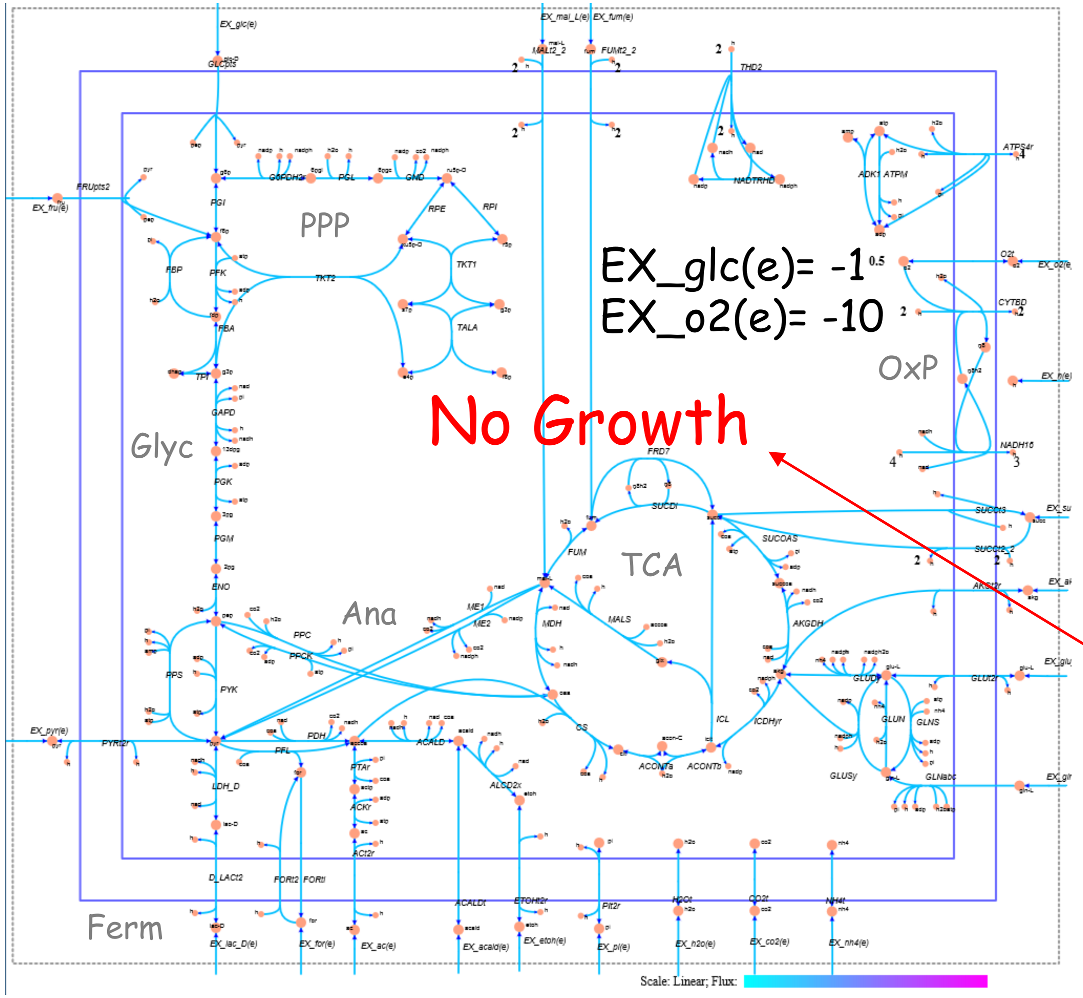
Note: Gurobi shadow prices are positive instead of the expected negative values

Phenotype Phase Plane Analysis Example (II)

Variables: EX_o2(e) & EX_glc(e)



Phenotype Phase Plane Analysis Example (Phase 1)



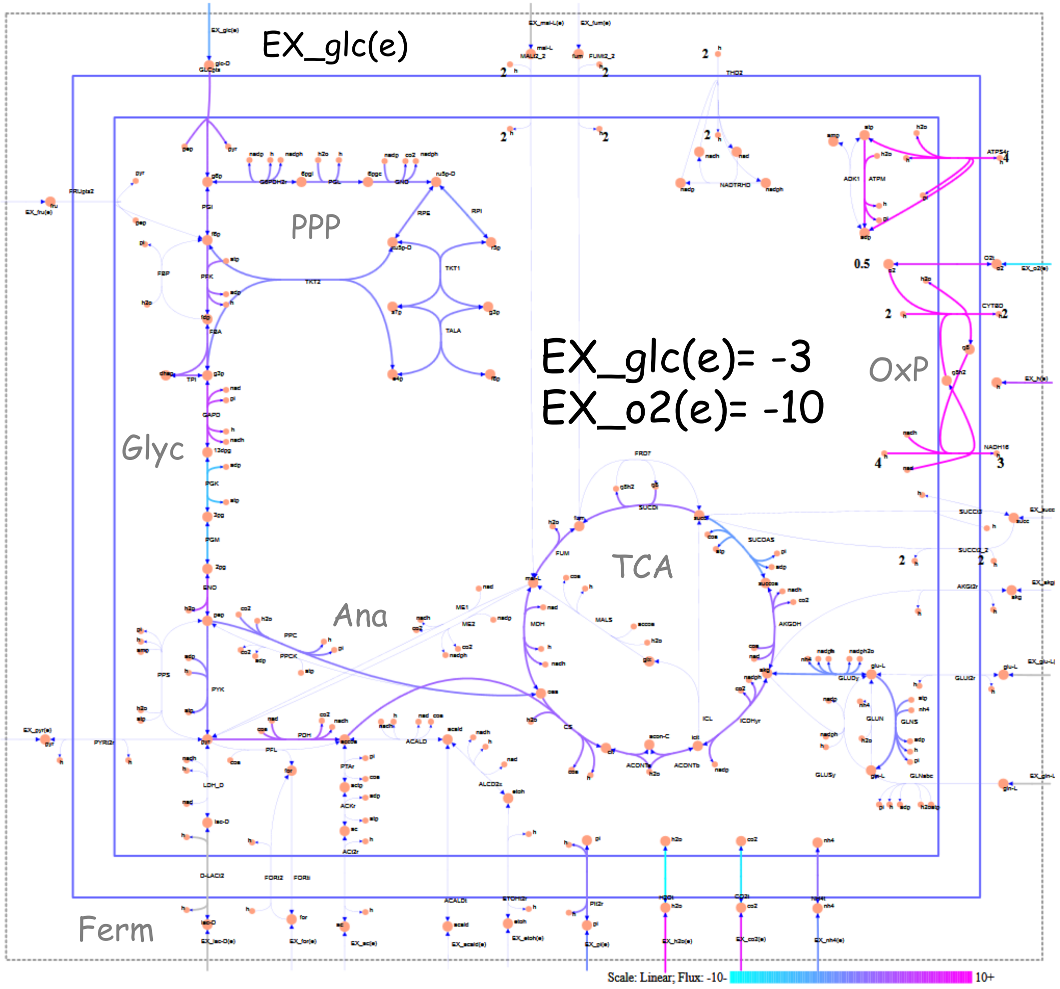
AerobicGlucoseBioMassPhase1.m

```
>> AerobicGlucoseBioMassPhase1

FBAsolution =
          full: []
           obj: []
         rcost: []
          dual: []
         slack: [72×1 double]
        solver: 'gurobi'
     algorithm: 'default'
          stat: 0
      origStat: 'INFEASIBLE'
          time: 0.0070
         basis: []
             f: 0
             x: []
```

No growth; not enough glucose

Phenotype Phase Plane Analysis Example (Phase 2)

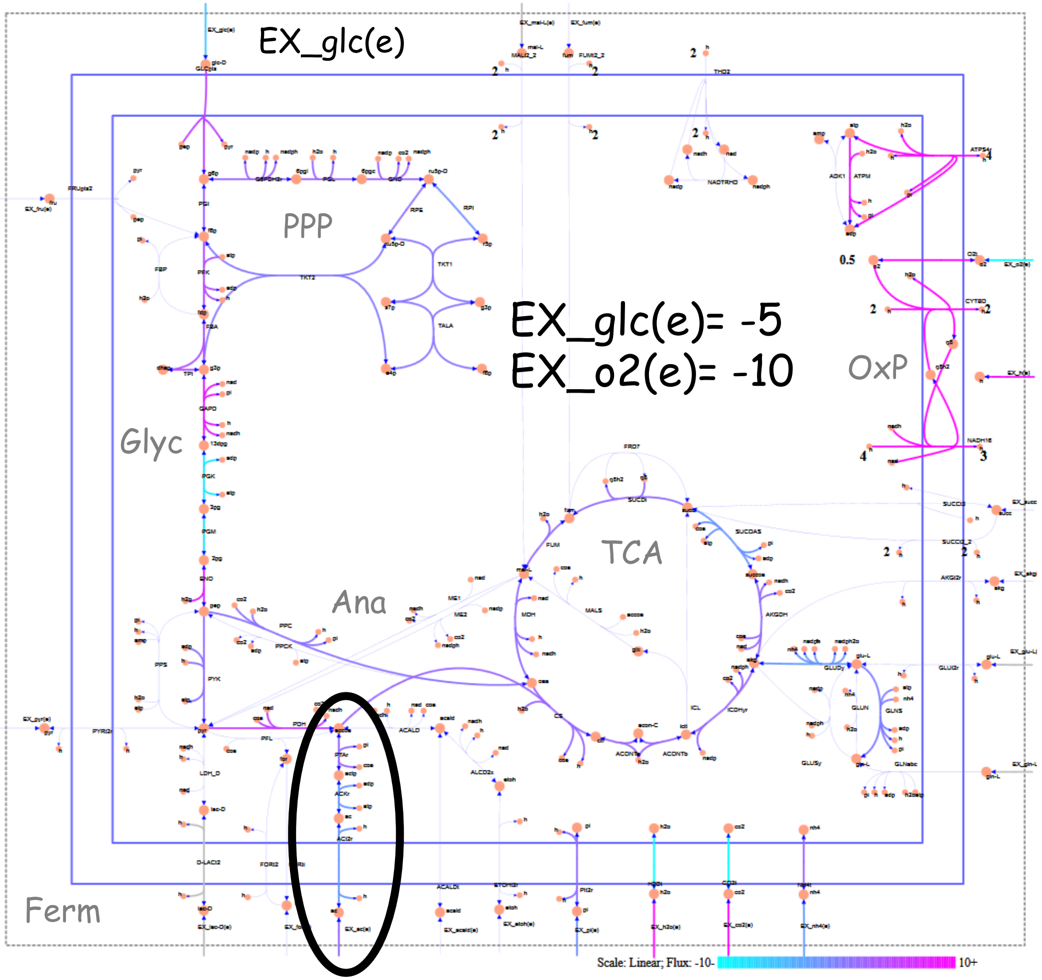


AerobicGlucoseBioMassPhase2.m

ACONTa	3.41586	FBA	2.81795	PGI	2.96248
ACONTb	3.41586	FUM	3.2184	PGK	-5.51348
AKGDH	2.69395	GAPD	5.51348	PGM	-5.23969
ATPM	8.39	GLCpts	3	PIt2r	0.673268
ATPS4r	21.278	GLNS	9.47587	PYK	2.14468
Biomass	0.183018	GLUDy	-0.951162	RPE	-0.131553
CO2t	-10.2116	GLUN	9.42907	RPI	-0.131553
CS	3.41586	H2Ot	-11.5448	SUCDi	3.2184
CYTBD	20	ICDHyr	2.89141	SUCOAS	-2.69395
ENO	5.23969	ICL	0.524456	TALA	-0.032741
EX_co2(e)	10.2116	MALS	0.524456	THD2	0.444093
EX_glc(e)	-3	MDH	3.74286	TKT1	-0.032741
EX_h2o(e)	11.5448	NADH16	16.7816	TKT2	-0.098811
EX_h(e)	3.67134	NH4t	0.997959	TPI	2.81795
EX_nh4(e)	-0.997959	O2t	10		
EX_o2(e)	-10	PDH	4.62623		
EX_pi(e)	-0.673268	PFK	2.81795		

Growth is limited by excess oxygen; not enough glucose to reduce all the oxygen and produce biomass optimally

Phenotype Phase Plane Analysis Example (Phase 3)

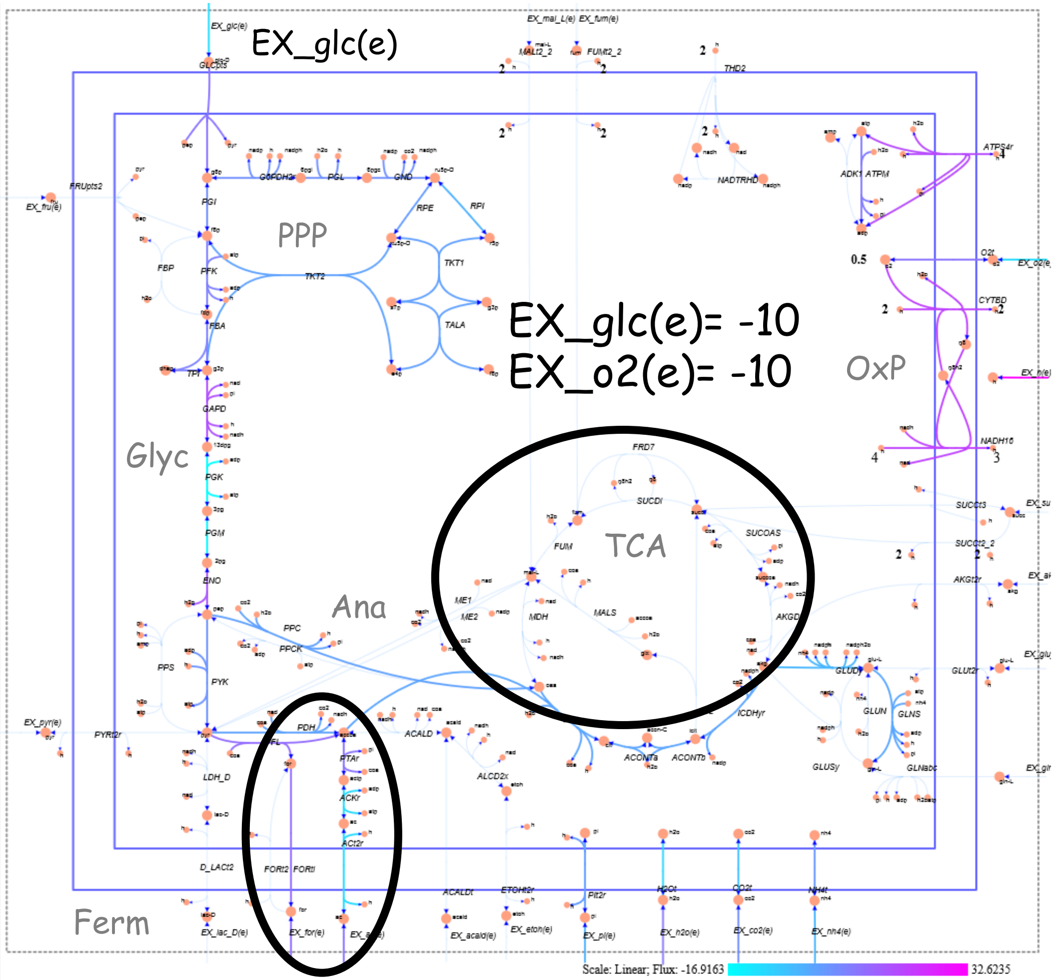


AerobicGlucoseBioMassPhase3.m

ACKr	-1.83668	EX_nh4(e)	-2.03666	PDH	5.34252
ACONTa	2.106	EX_o2(e)	-10	PFK	3.84494
ACONTb	2.106	EX_pi(e)	-1.37402	PGI	2.57284
ACt2r	-1.83668	FBA	3.84494	PGK	-8.22357
AKGDH	1.70302	FUM	1.70302	PGL	2.35059
ATPM	8.39	G6PDH2r	2.35059	PGM	-7.66481
ATPS4r	21.5061	GAPD	8.22357	PIt2r	1.37402
Biomass	0.373508	GLCpts	5	PPC	1.07032
CO2t	-10.4318	GLNS	0.095506	PTAr	1.83668
CS	2.106	GLUDy	-1.94116	PYK	1.40059
CYTBD	20	GND	2.35059	RPE	1.29858
ENO	7.66481	H2Ot	-13.1526	RPI	-1.05201
EX_ac(e)	1.83668	ICDHyr	2.106	SUCDi	1.70302
EX_co2(e)	10.4318	MDH	1.70302	SUCOAS	-1.70302
EX_glc(e)	-5	NADH16	18.297	TALA	0.71671
EX_h2o(e)	13.1526	NH4t	2.03666	TKT1	0.71671
EX_h(e)	9.32925	O2t	10	TKT2	0.581873
				TPI	3.84494

Not enough oxygen to fully oxidize glucose; acetate produced through fermentation

Phenotype Phase Plane Analysis Example (Phase 4)

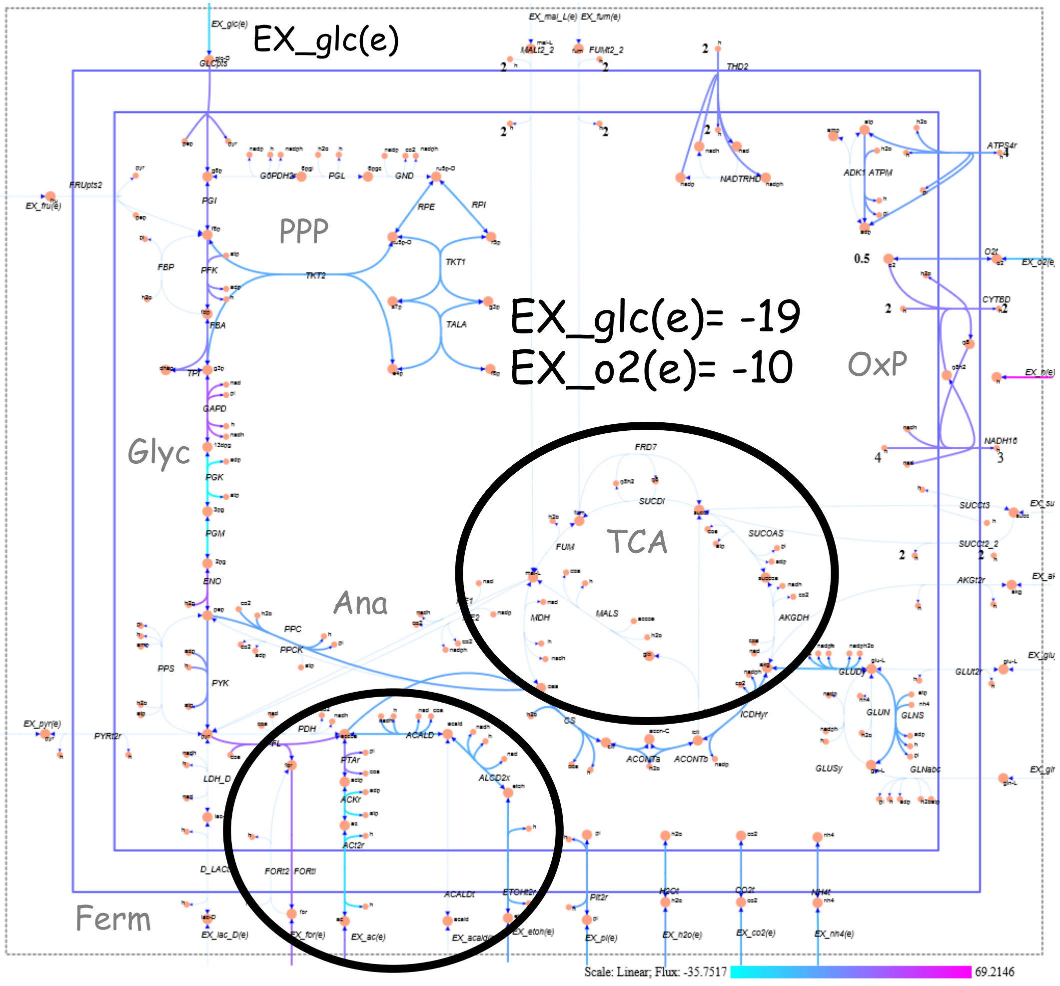


AerobicGlucoseBioMassPhase4.m

ACKr	-9.90568	EX_o2(e)	-10	PGI	5.09263
ACONTa	0.60316	EX_pi(e)	-2.05658	PGK	-16.9163
ACONTb	0.60316	FBA	7.84632	PGL	4.79277
ACt2r	-9.90568	FORti	11.5033	PGM	-16.0799
ATPM	8.39	G6PDH2r	4.79277	PIt2r	2.05658
ATPS4r	18.8064	GAPD	16.9163	PPC	1.60202
Biomass	0.559051	GLCpts	10	PTAr	9.90568
CO2t	-4.89467	GLNS	0.142949	PYK	4.18773
CS	0.60316	GLUDy	-2.90544	RPE	2.79333
CYTBD	20	GND	4.79277	RPI	-1.99944
ENO	16.0799	H2Ot	-8.96702	TALA	1.49758
EX_ac(e)	9.90568	ICDHyr	0.60316	TKT1	1.49758
EX_co2(e)	4.89467	NADH16	20	TKT2	1.29576
EX_for(e)	11.5033	NH4t	3.04839	TPI	7.84632
EX_glc(e)	-10	O2t	10		
EX_h2o(e)	8.96702	PDH	1.10076		
EX_h(e)	32.6235	PFK	7.84632		
EX_nh4(e)	-3.04839	PFL	11.5033		

Not enough oxygen to fully oxidize glucose; acetate and formate are produced and secreted

Phenotype Phase Plane Analysis Example (Phase 5)



AerobicGlucoseBioMassPhase5.m

ACALD	-2.12502	EX_glc(e)	-19	PFL	29.2279
ACKr	-23.0206	EX_h2o(e)	2.52474	PGI	18.8266
ACONTa	0.912497	EX_h(e)	69.2146	PGK	-35.7517
ACONTb	0.912497	EX_nh4(e)	-4.61179	PGM	-34.4864
ACt2r	-23.0206	EX_o2(e)	-10	PIt2r	3.11132
ALCD2x	-2.12502	EX_pi(e)	-3.11132	PPC	2.42363
ATPM	8.39	FBA	18.1587	PTAr	23.0206
ATPS4r	5.95415	FORti	29.2279	PYK	12.6238
Biomass	0.845766	GAPD	35.7517	RPE	-0.607936
CO2t	1.51113	GLCpts	19	RPI	-0.607936
CS	0.912497	GLNS	0.216262	TALA	-0.151307
CYTBD	20	GLUDy	-4.39553	THD2	14.5016
ENO	34.4864	H2Ot	-2.52474	TKT1	-0.151307
ETOHt2r	-2.12502	ICDHyr	0.912497	TKT2	-0.456629
EX_ac(e)	23.0206	NADH16	20	TPI	18.1587
EX_co2(e)	-1.51113	NH4t	4.61179		
EX_etoh(e)	2.12502	O2t	10		
EX_for(e)	29.2279	PFK	18.1587		

Not enough oxygen to fully oxidize glucose; acetate, formate and ethanol are produced and secreted.

Phenotype Phase Plane Analysis Example (VI)

Variables: EX_o2(e) & EX_glc(e)

Phase 3

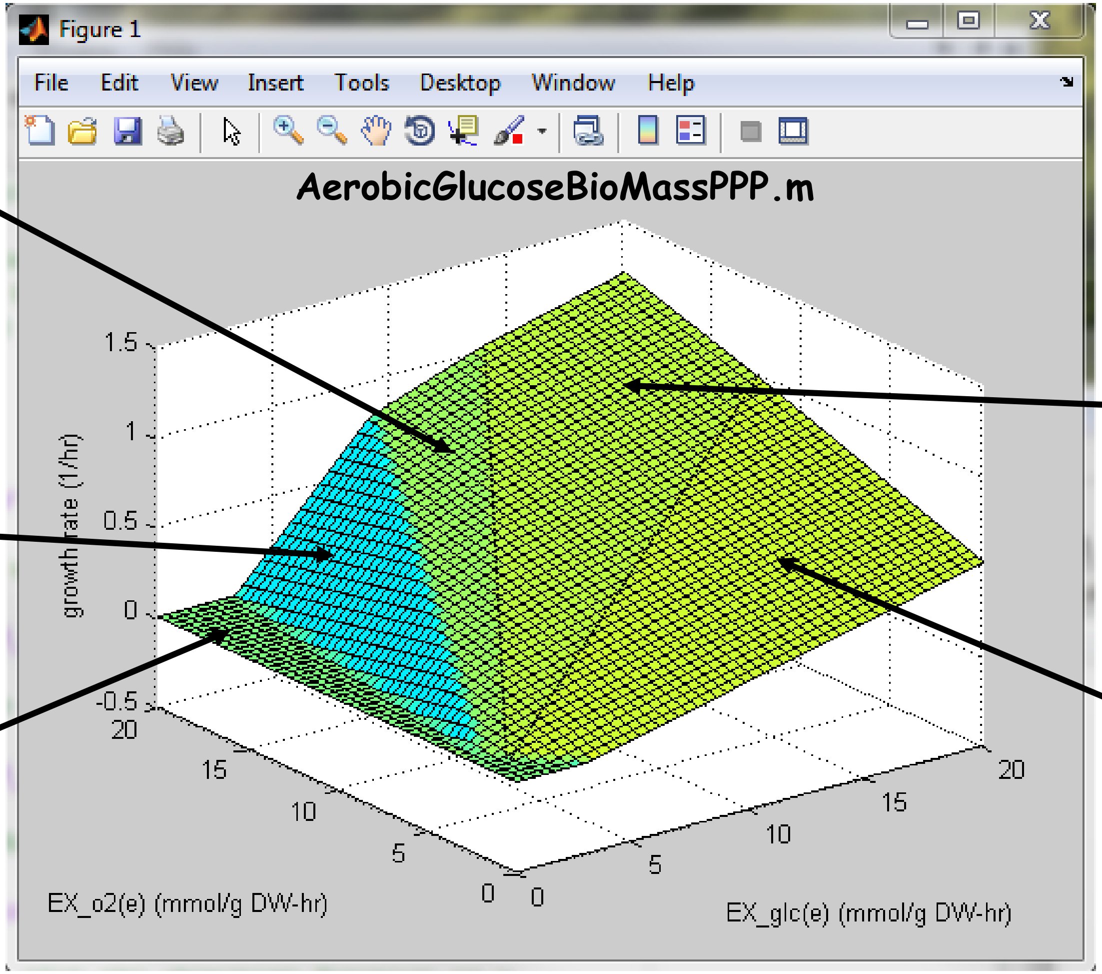
Not enough oxygen to fully oxidize glucose; acetate produced through fermentation

Phase 2

Growth is limited by excess oxygen; not enough glucose to reduce all the oxygen and produce biomass optimally

Phase 1

No growth; not enough glucose



Phase 4

Not enough oxygen to fully oxidize glucose; acetate and formate are produced and secreted

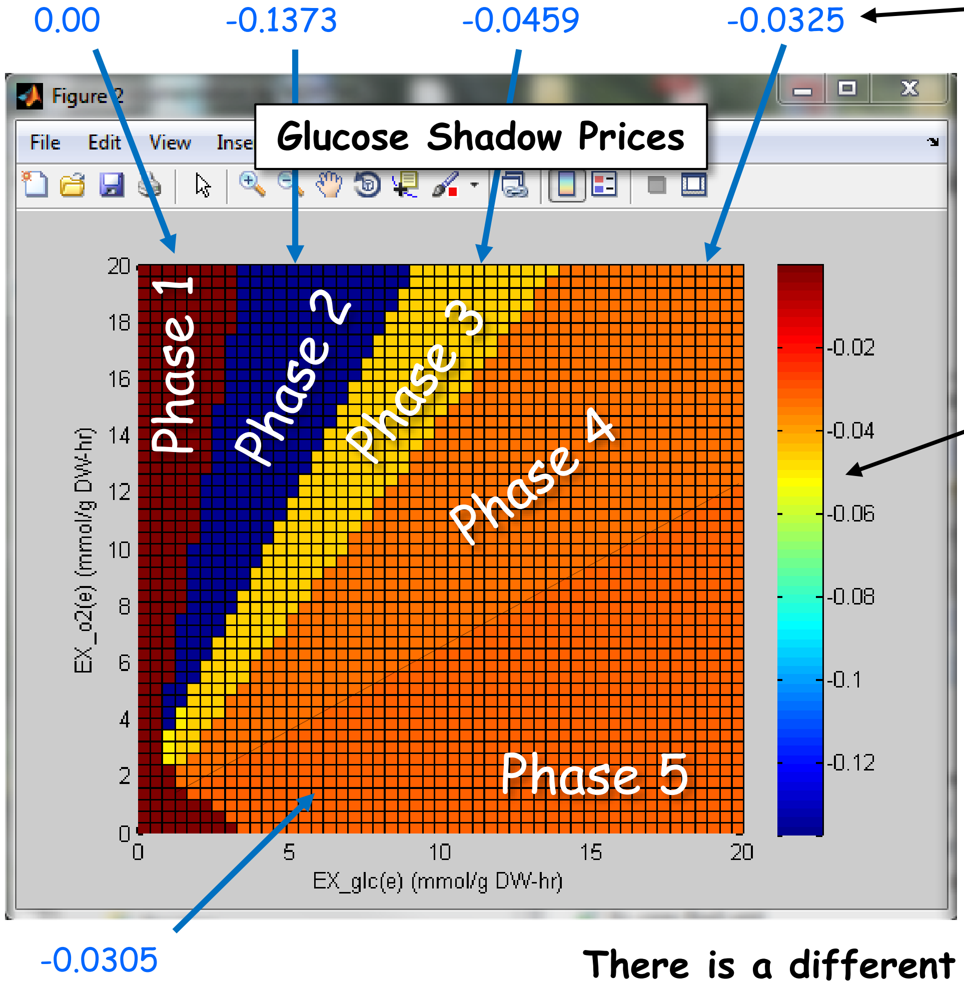
Phase 5

Not enough oxygen to fully oxidize glucose; acetate, formate and ethanol are produced and secreted.

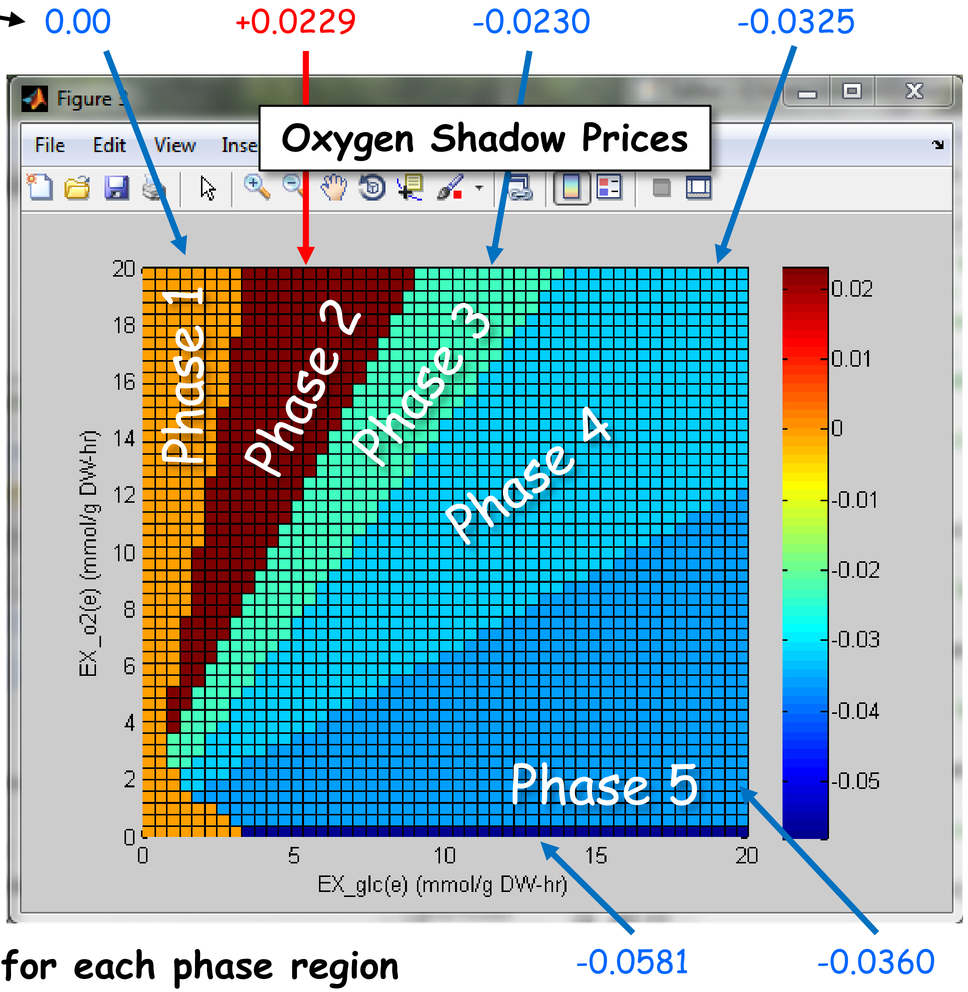
Orth, J. D., I. Thiele, et al. (2010). "What is flux balance analysis?" Supplementary Tutorial, Nature biotechnology 28(3): 245-248. (Supplementary Examples 4 & 5)

Phenotype Phase Plane Analysis Example (VII)

Gurobi shadow prices multiplied by -1



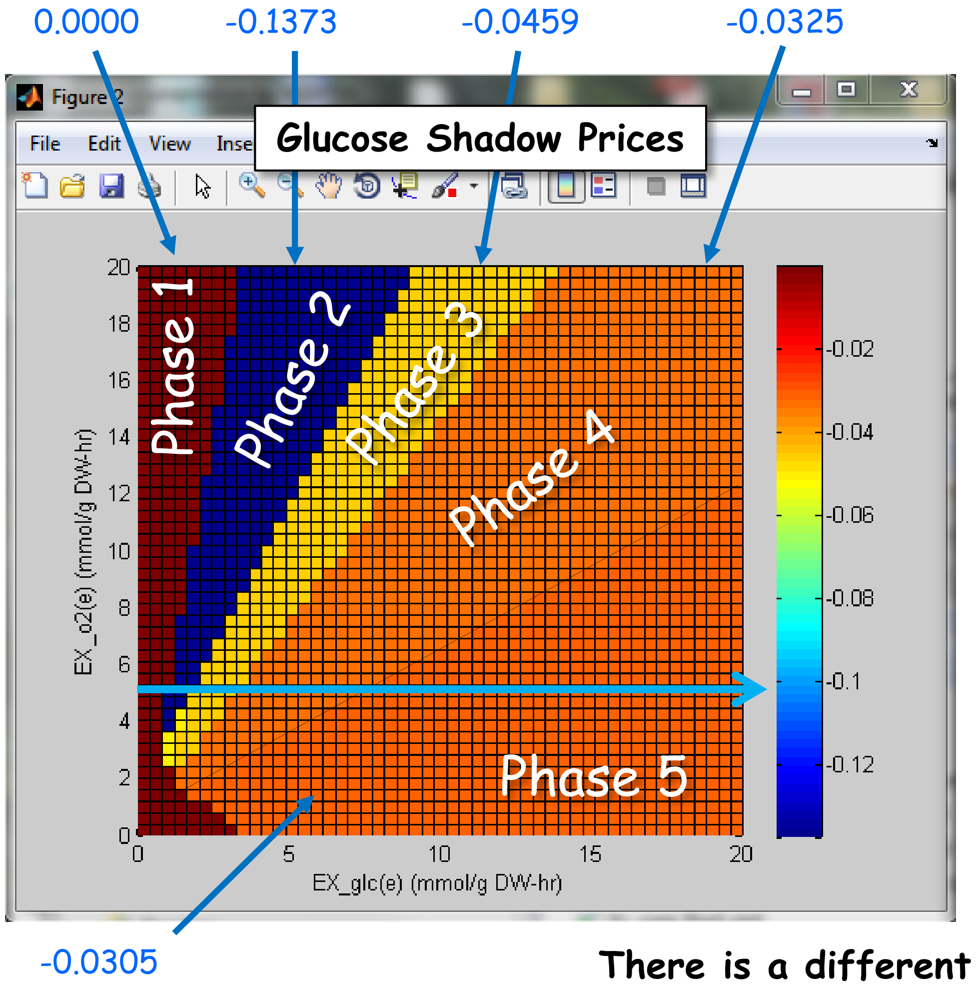
Gurobi positive shadow prices



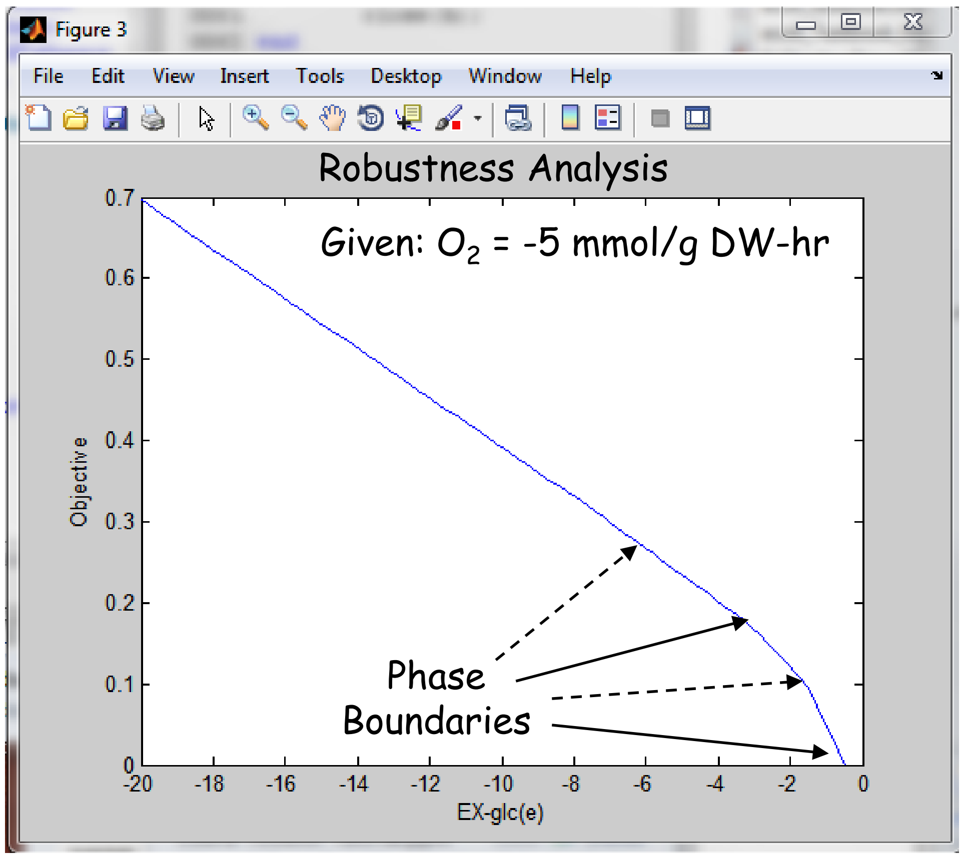
There is a different phenotype for each phase region

Phenotype Phase Plane Analysis Example (VIII)

(Robustness Analysis setting O_2 = -5 mmol/g DW-hr)



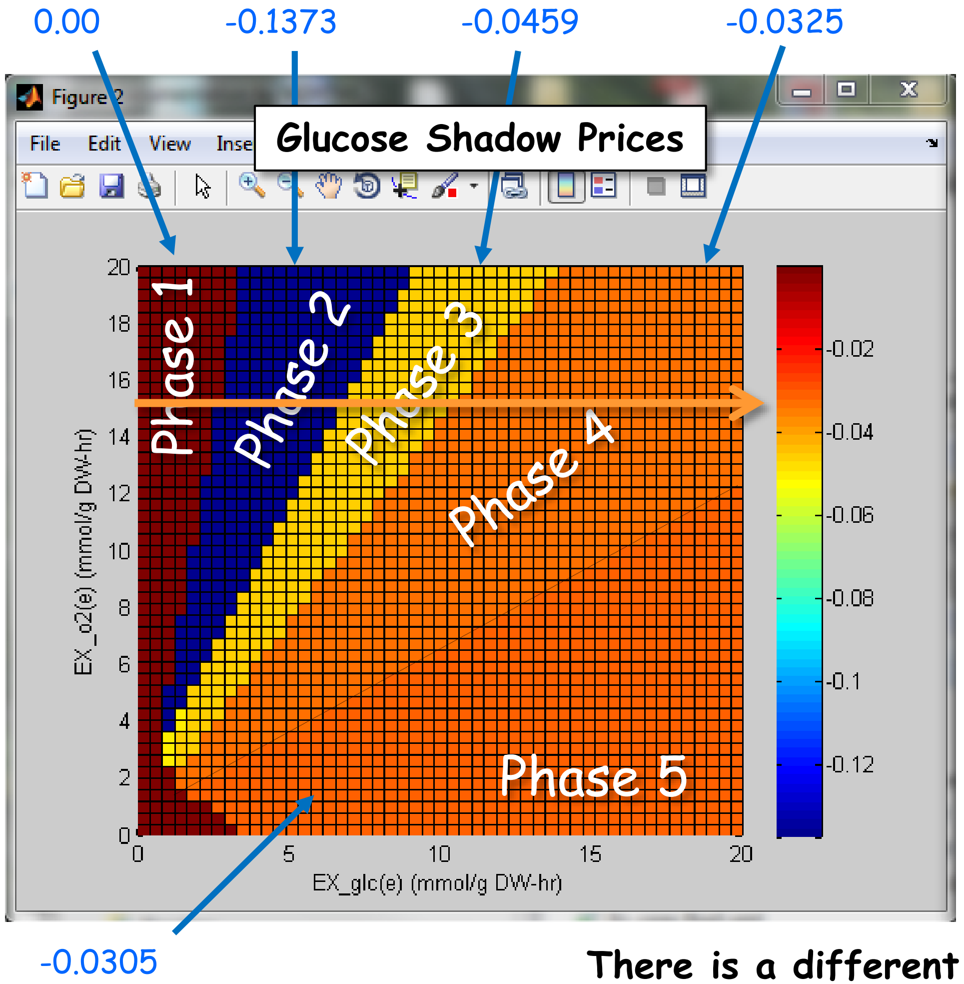
AerobicGlucoseBioMassRA_glc.m



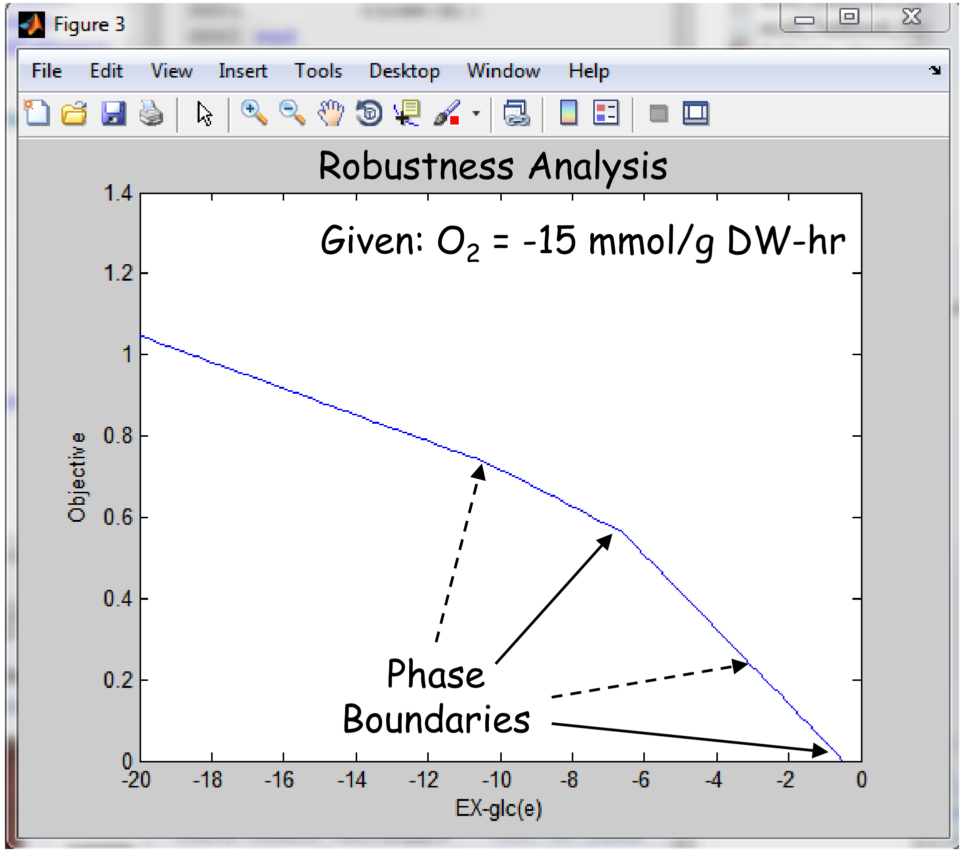
There is a different phenotype for each phase region

Phenotype Phase Plane Analysis Example (IX)

(Robustness Analysis setting O_2 = -15 mmol/g DW-hr)



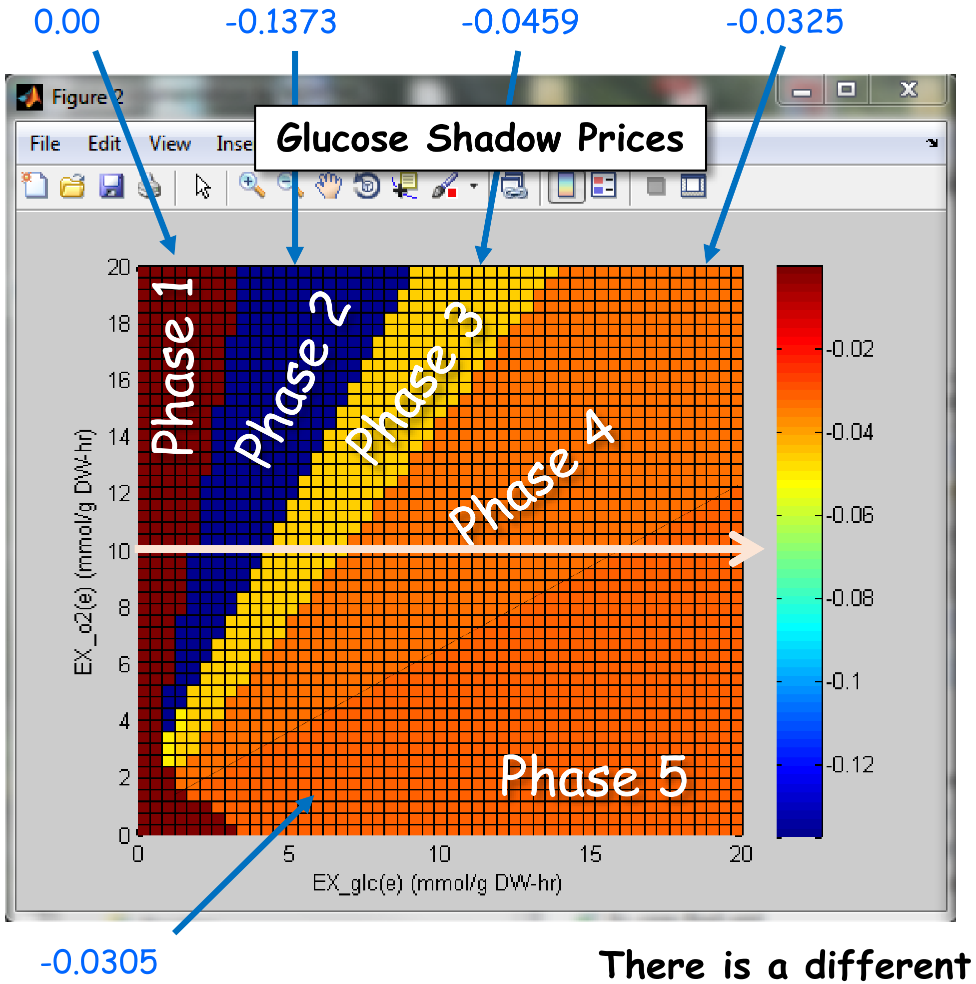
AerobicGlucoseBioMassRA_glc.m

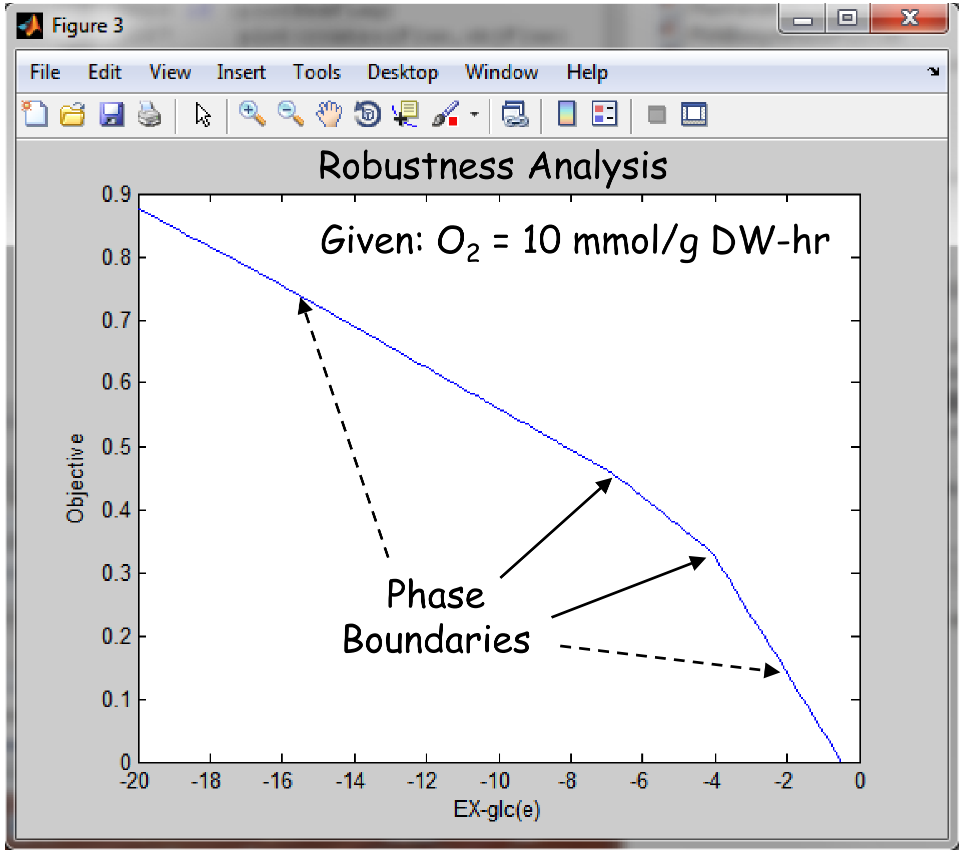


There is a different phenotype for each phase region

Phenotype Phase Plane Analysis Example (X)

(Robustness Analysis setting O_2 = 10 mmol/g DW-hr)

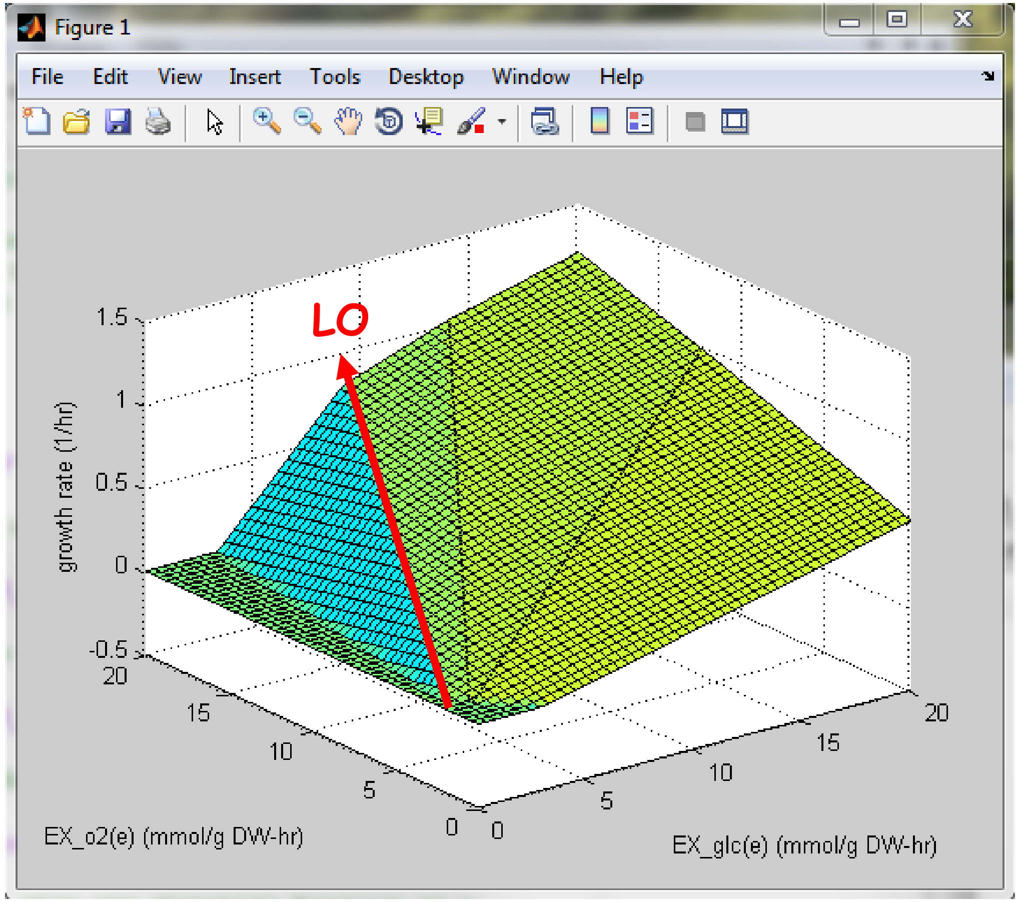




There is a different phenotype for each phase region

Line of Optimality

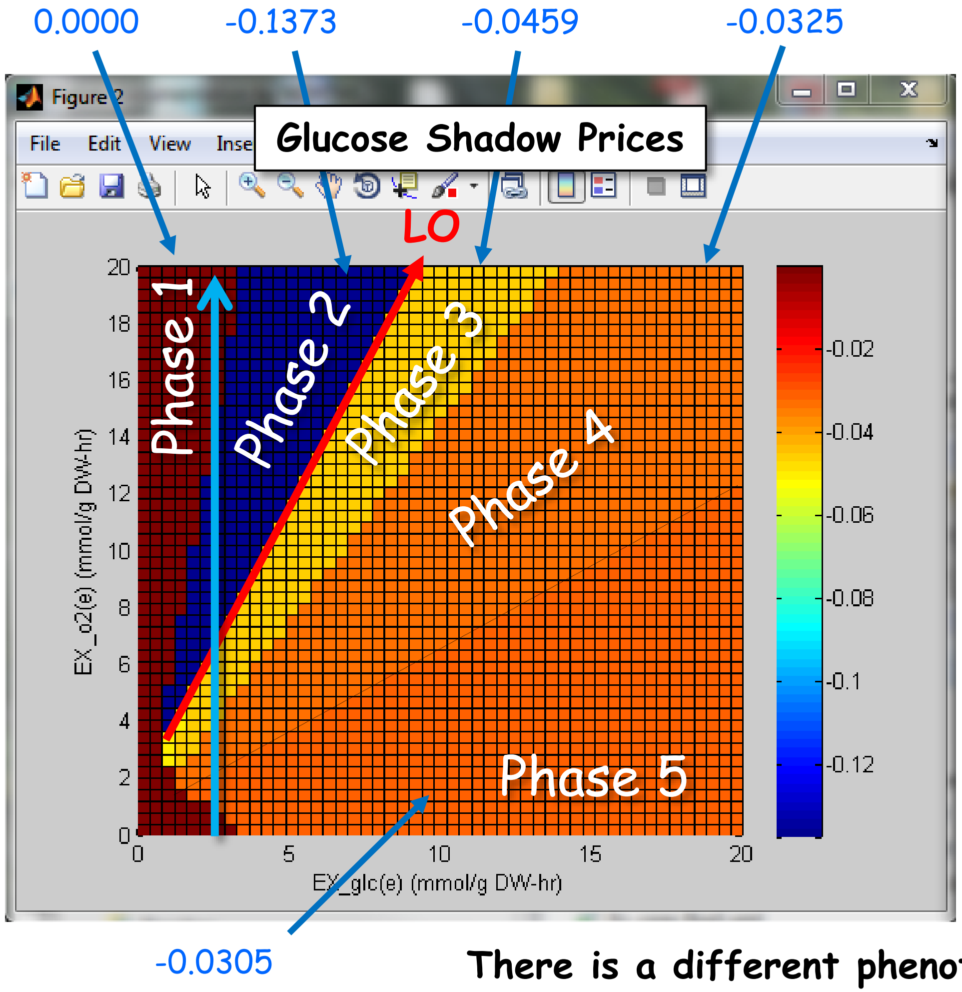
- The line of optimality (LO) is defined as a line representing the optimal relation between the two metabolic fluxes used to create a phenotype phase plane.
- The line of optimality is determined by specifying an uptake rate of the substrate along the x-axis and then allowing any value for the flux along the y-axis. Linear Programming can then be used to calculate the optimal value of the objective as a function of the y-axis flux. Once the objective is determined, the corresponding flux value for the y-axis is used to plot the line of optimality (LO).
- The LO defines the optimal utilization of the metabolic pathways without limitations on the availability of the substrates.



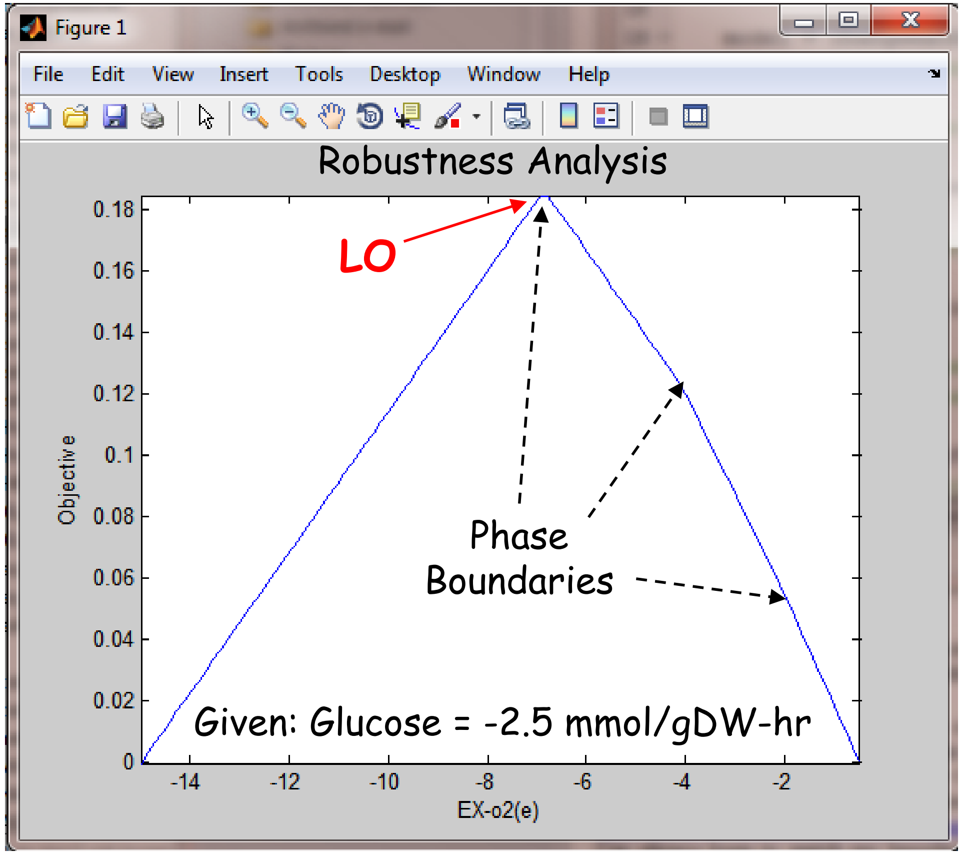
Edwards, J. S., R. U. Ibarra, et al. (2001). "In silico predictions of Escherichia coli metabolic capabilities are consistent with experimental data." Nat Biotechnol 19(2): 125-130.

Phenotype Phase Plane Analysis Example (XI)

(Robustness Analysis setting Glucose = -2.5 mmol/gDW-hr)



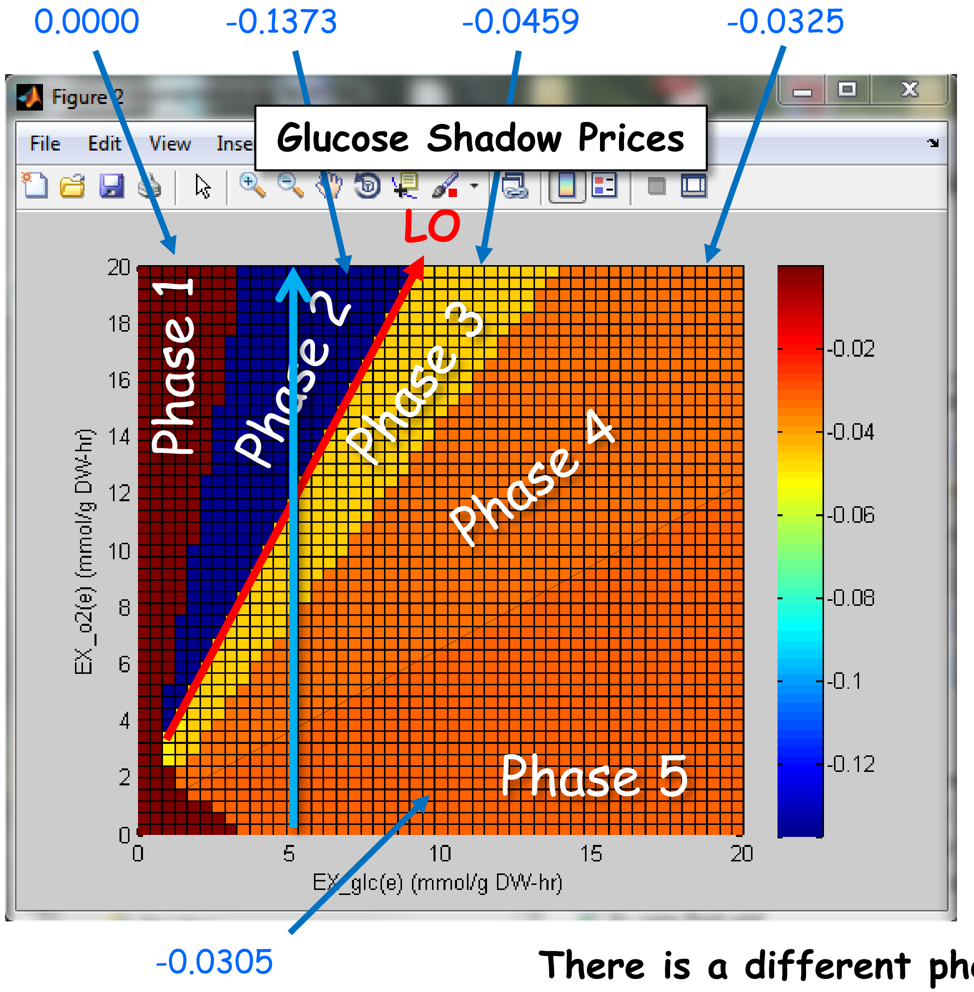
AerobicGlucoseBioMassRA_o2.m

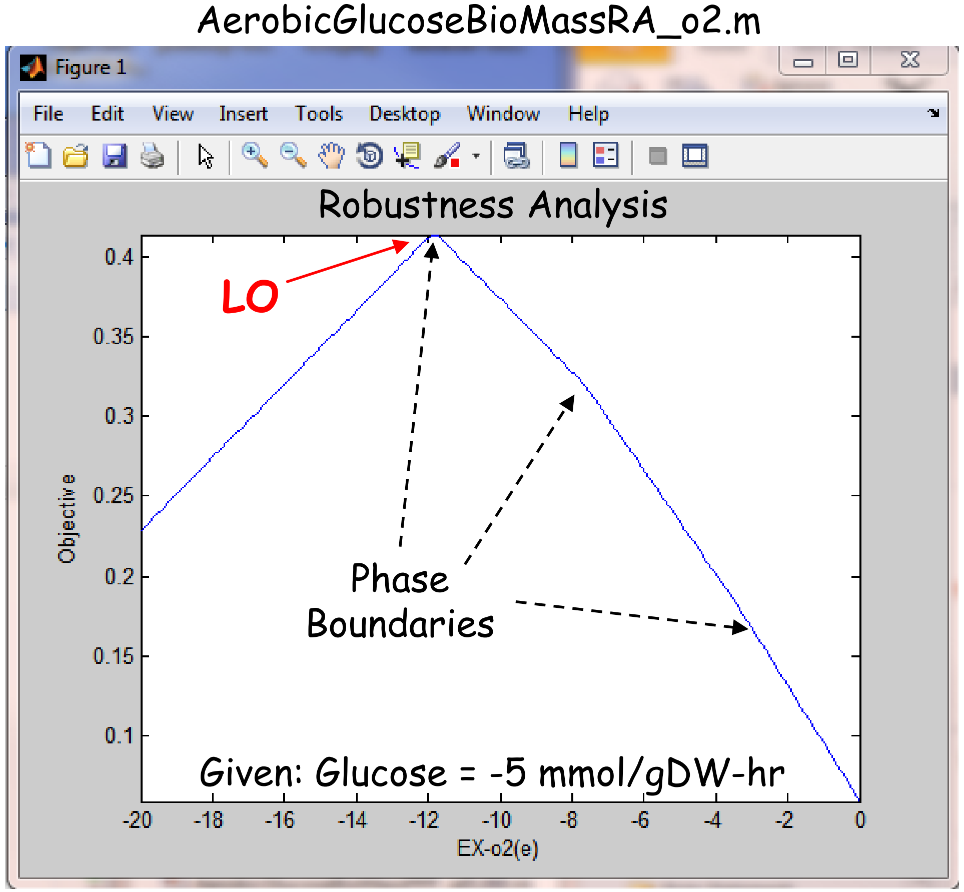


There is a different phenotype for each phase region

Phenotype Phase Plane Analysis Example (XII)

(Robustness Analysis setting Glucose = -5 mmol/gDW-hr)





There is a different phenotype for each phase region

Phenotype Phase Plane Analysis Example (XIII)

Variables: EX_o2(e) & EX_glc(e)

Phase 3

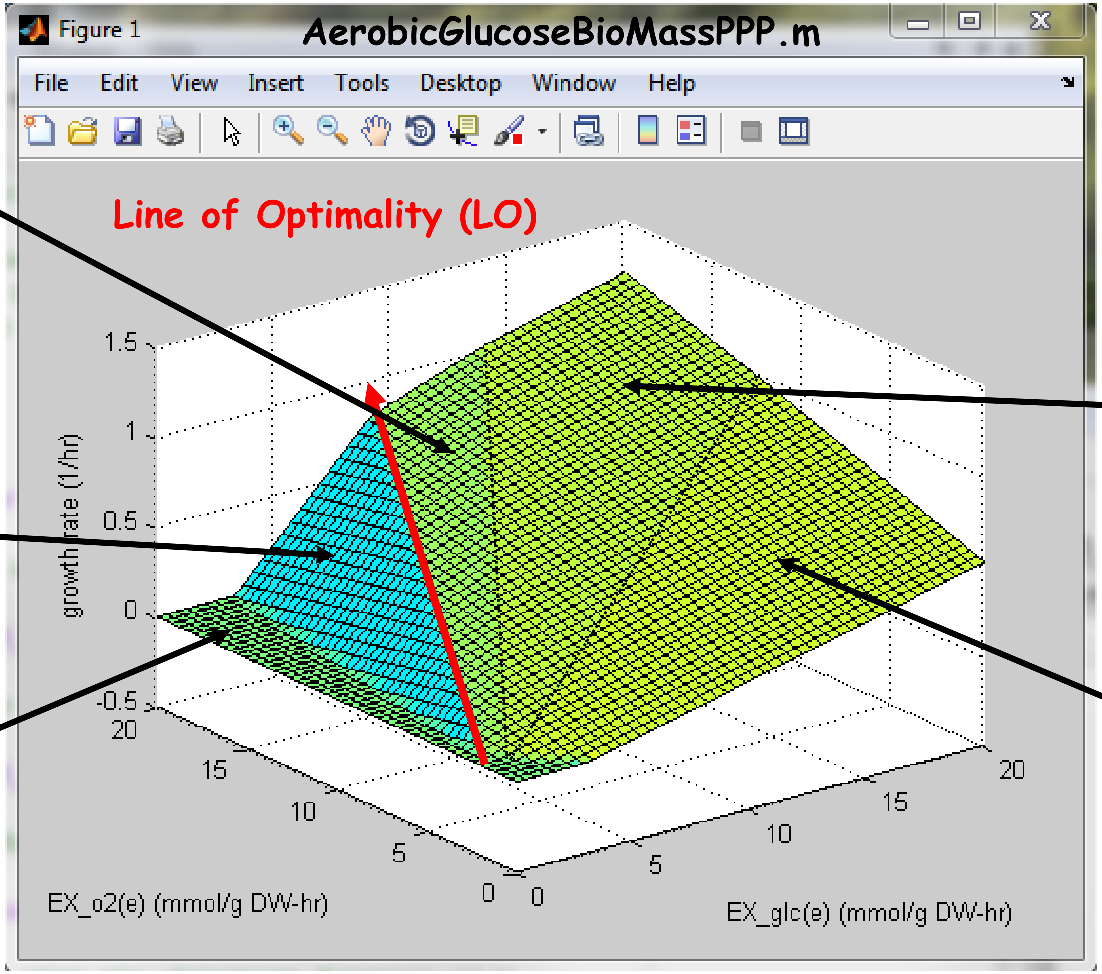
Not enough oxygen to fully oxidize glucose; acetate produced through fermentation

Phase 2

Growth is limited by excess oxygen; not enough glucose to reduce all the oxygen and produce biomass optimally

Phase 1

No growth; not enough glucose



Phase 4

Not enough oxygen to fully oxidize glucose; acetate and formate are produced and secreted

Phase 5

Not enough oxygen to fully oxidize glucose; acetate, formate and ethanol are produced and secreted.

Orth, J. D., I. Thiele, et al. (2010). "What is flux balance analysis?" Supplementary Tutorial, Nature biotechnology 28(3): 245-248. (Supplementary Examples 4 & 5)

Ratio of Relative Shadow Prices

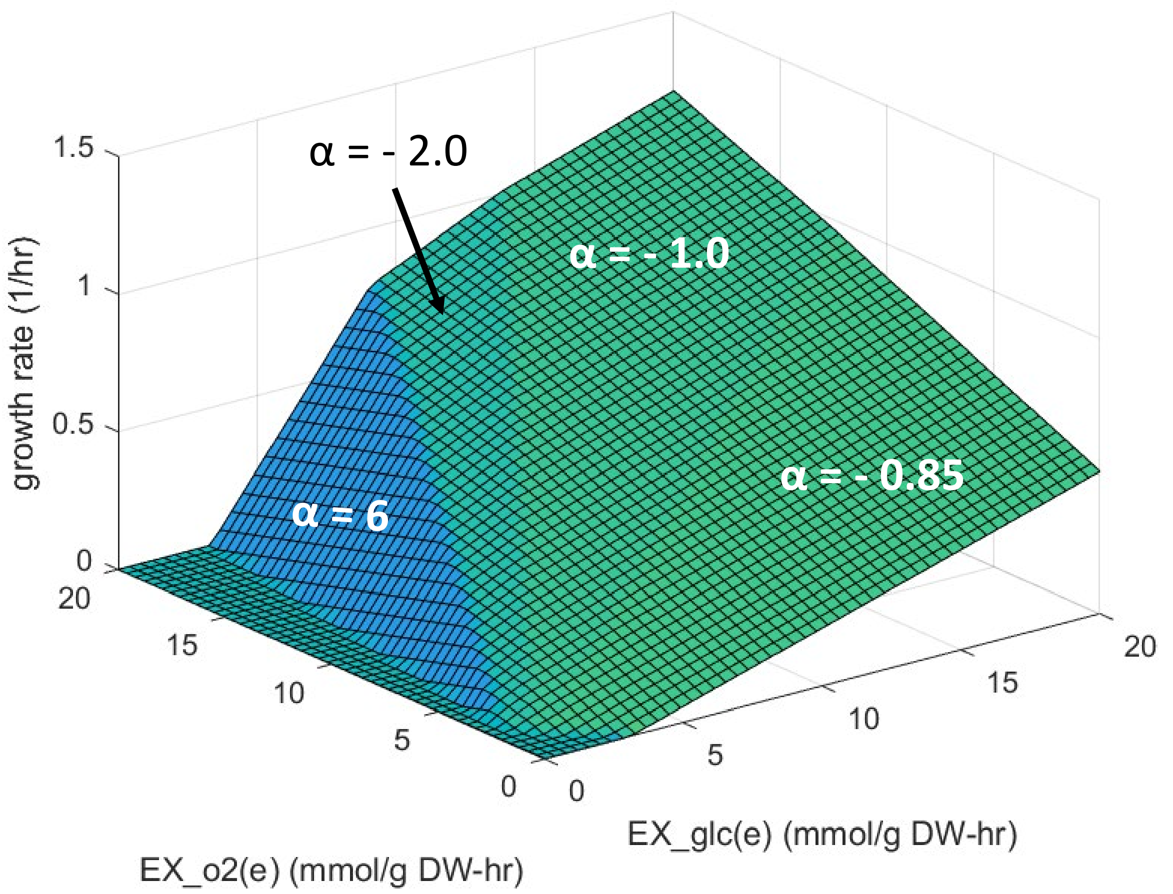
- The regions in the phenotype phase plance (PhPP) can be defined based on the contributions of the two parameters represented on the x and y axes to the objective function. To facilitate such an interpretation, we define the ratio of the relative shadow prices for the two variables on the axes of the PhPP:

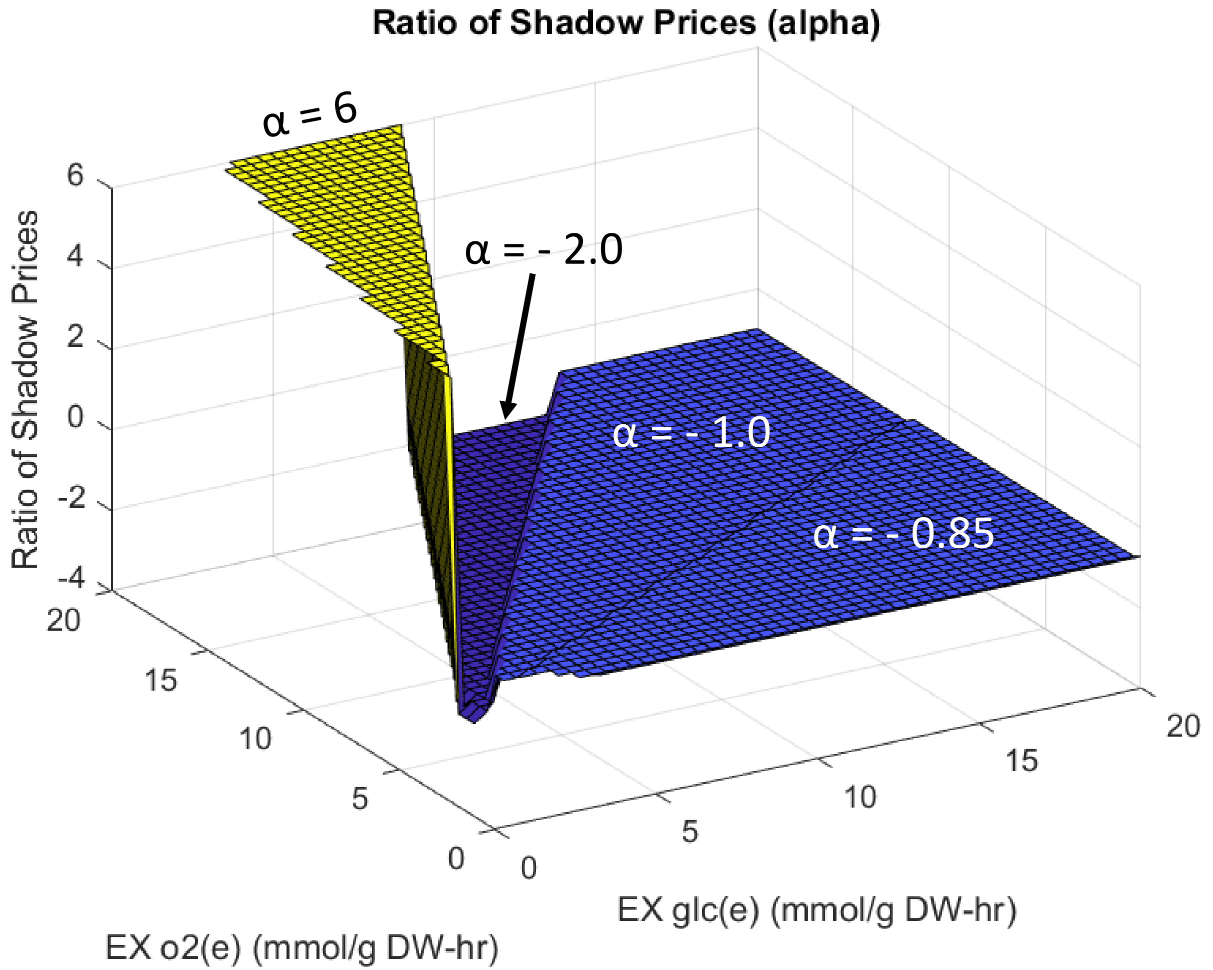
$$\alpha = -\frac{\pi_x}{\pi_y}$$

- where π is the shadow price and x and y refer to the variables on the x and y axes.
- The negative sign on α is introduced in anticipation of its interpretation. The ratio α is the relative change in the objective function for changes in the two key exchange fluxes. In order for the objective function to remain constant, an increase in one of the exchange fluxes will be accompanied by a decrease in the other, and thus we introduce the negative sign on the definition of α.
- The parameter α is thus the slope of a line in the PhPP along which the value of the objective function is a constant. This line is called an isocline.

Palsson, Bernhard Ø., *Systems Biology: Constraint-Based Reconstruction and Analysis*. Cambridge University Press, page 370

Growth-rate & Ratio of Shadow Prices

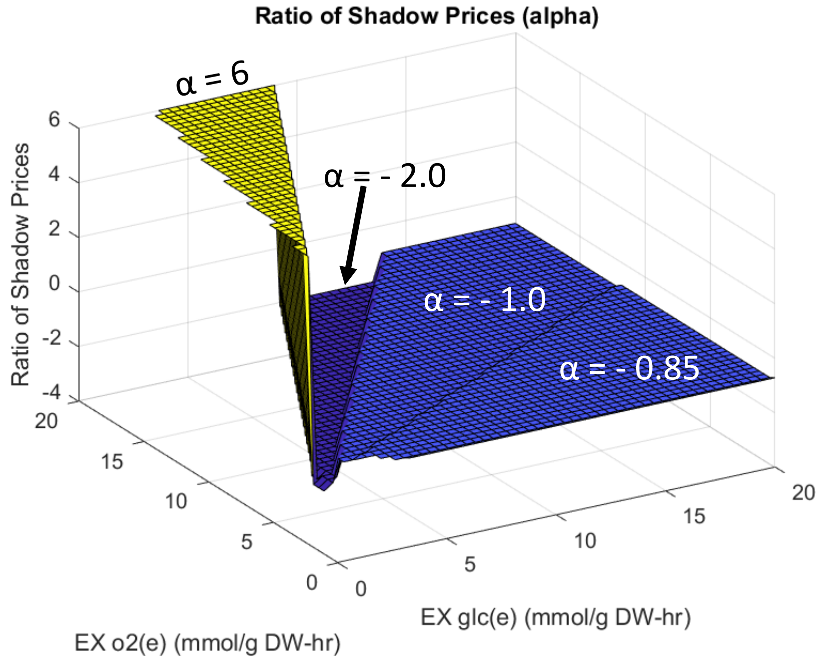




Characteristics of Phases in a Phenotype Phase Plane

The slope of the isoclines within each phase of the phenotype phase plane (PhPP) is calculated from the shadow prices. Thus, the slope of the isoclines will be different in each region of the PhPP. Based on these considerations we identify four types of regions on the PhPP:

1. In phases where the α value is negative, there is dual limitation of the substrates. Based on the absolute value of α, the substrate with a greater contribution toward obtaining the objective can be identified. If the absolute value of α is greater than unity, the substrate along the x-axis is more valuable toward obtaining the objective, whereas if the absolute value of α is less than unity, the substrate along the y-axis is more valuable to the objective.
2. The phases where the isoclines are either horizontal or vertical are phases of single substrate limitation; the α value in these phases will be zero or infinite, respectively. These phases arise when the shadow price for one of the substrates goes to zero, and thus has no value to the cell
3. Phases in the PhPP can also have a positive α value, termed 'futile' phases. In these phases, one of the substrates is inhibitory toward obtaining the objective function, and this substrate will have a positive shadow price. The metabolic operation in this phase is wasteful, in that it consumes substrate that is not needed to improve the objective. Phases with positive α values are expected to be phenotypically unstable, i.e., a cell would not be expected to choose a state in such a region. Under selection pressure, cells would move their phenotype state out of the phase.
4. Finally, due to stoichiometric limitations, there are infeasible steady-state phases in the PhPP. If the substrates are taken up at the rates represented by these points, the metabolic network is not able to obey the mass, energy, and redox constraints while generating biomass.

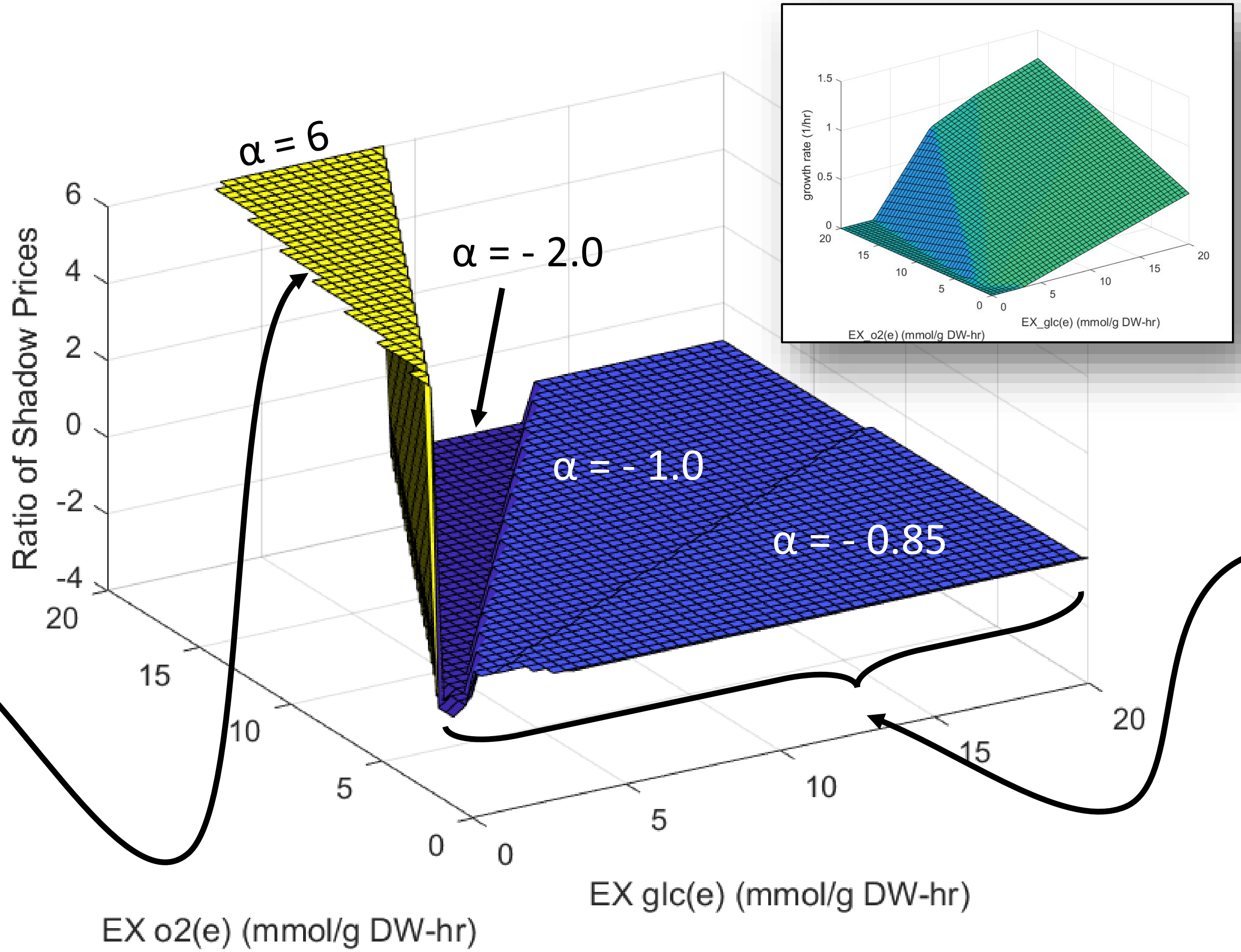


Palsson, Bernhard Ø.. Systems Biology: Constraint-Based Reconstruction and Analysis. Cambridge University Press. Page 370

Ratio of Shadow Prices (α)

α > 0

Phases in the PhPP can also have a **positive α value**, termed 'futile' phases. In these phases, one of the substrates is inhibitory toward obtaining the objective function, and this substrate will have a positive shadow price. The metabolic operation in this phase is wasteful, in that it consumes substrate that is not needed to improve the objective. Phases with positive α values are expected to be **phenotypically unstable**, i.e., a cell would not be expected to choose a state in such a region. **Under selection pressure, cells would move their phenotype state out of the phase.**



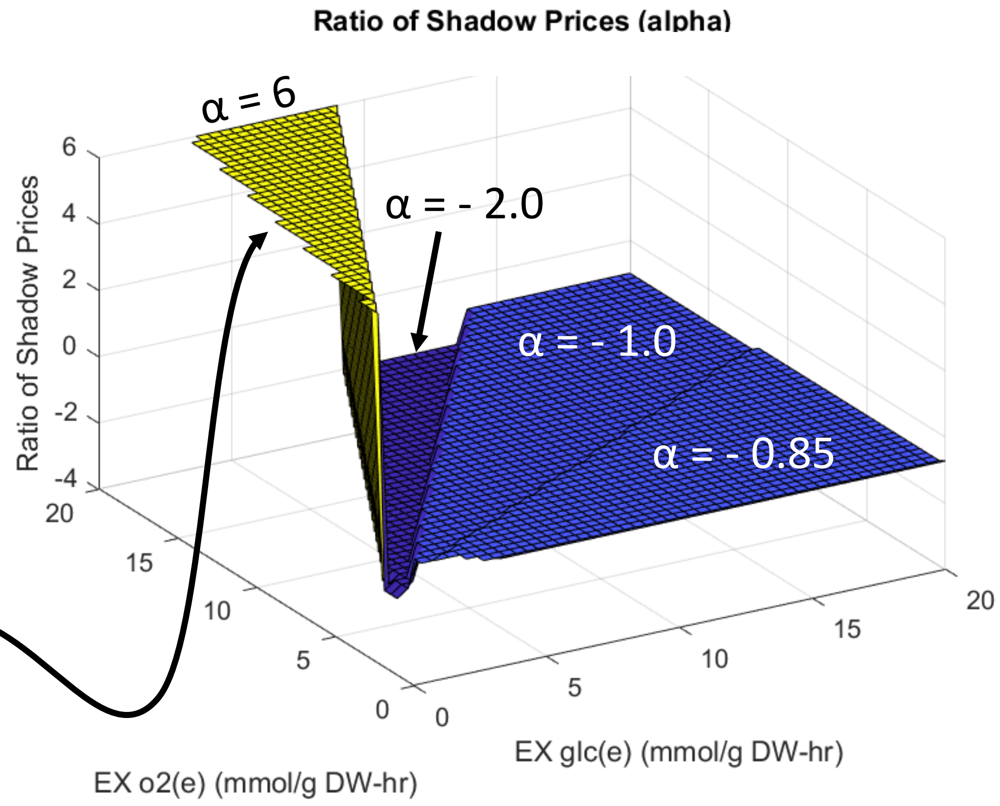
α < 0

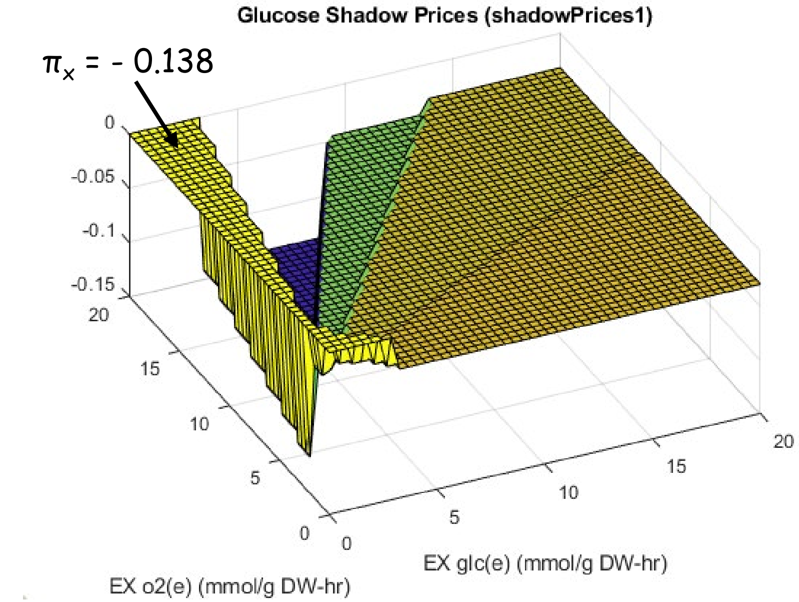
In phases where the **α value is negative**, there is **dual limitation** of the substrates. Based on the absolute value of α, the substrate with a greater contribution toward obtaining the objective can be identified. If the absolute value of α is greater than unity, the substrate along the x-axis is more valuable toward obtaining the objective, whereas if the absolute value of α is less than unity, the substrate along the y-axis is more valuable to the objective.

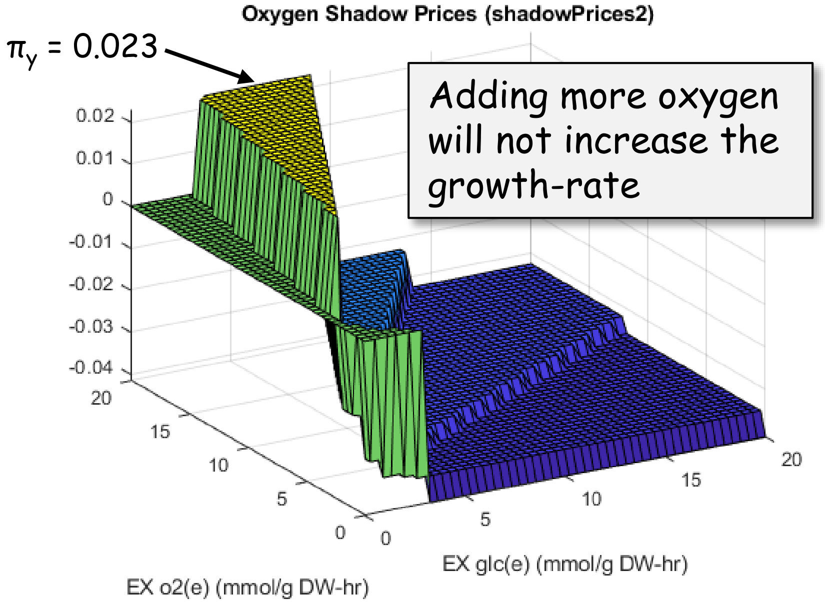
Ratio of Shadow Prices (α > 0)

α > 0

Phases in the PhPP can also have a **positive α value**, termed 'futile' phases. In these phases, ***one of the substrates is inhibitory toward obtaining the objective function, and this substrate will have a positive shadow price***. The metabolic operation in this phase is **wasteful**, in that it consumes substrate that is not needed to improve the objective. Phases with positive α values are expected to be **phenotypically unstable**, i.e., a cell would not be expected to choose a state in such a region. **Under selection pressure, cells would move their phenotype state out of the phase.**







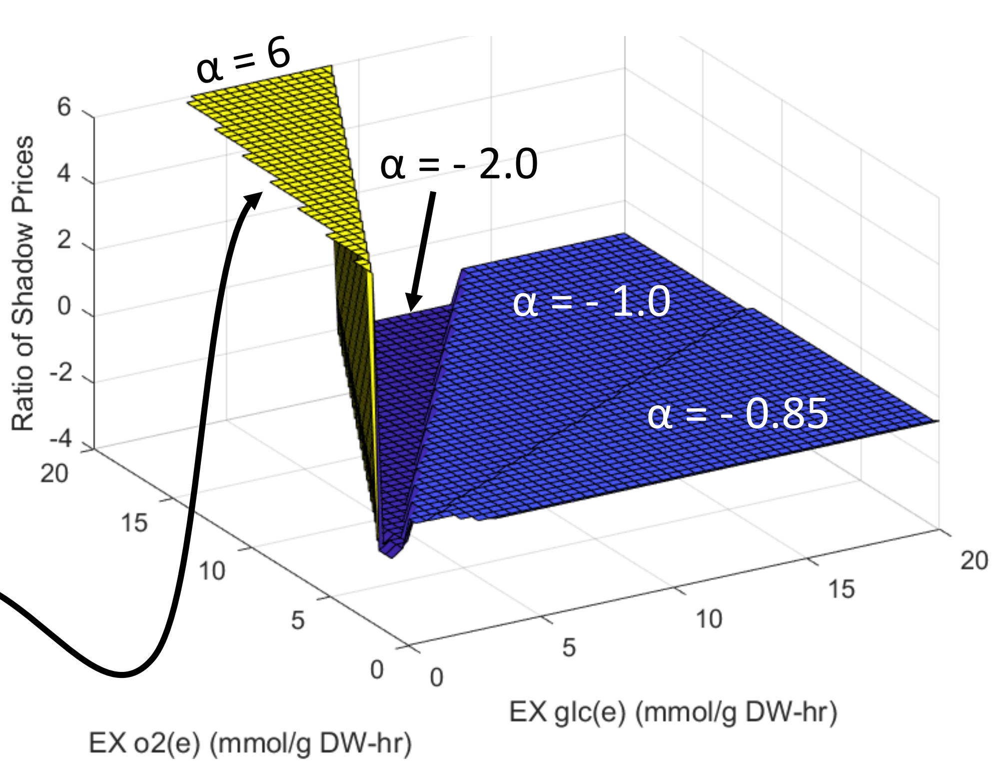
Growth is limited by excess oxygen; not enough glucose to reduce all the oxygen and produce biomass optimally

AerobicGlucoseBioMassPPP_alpha.m

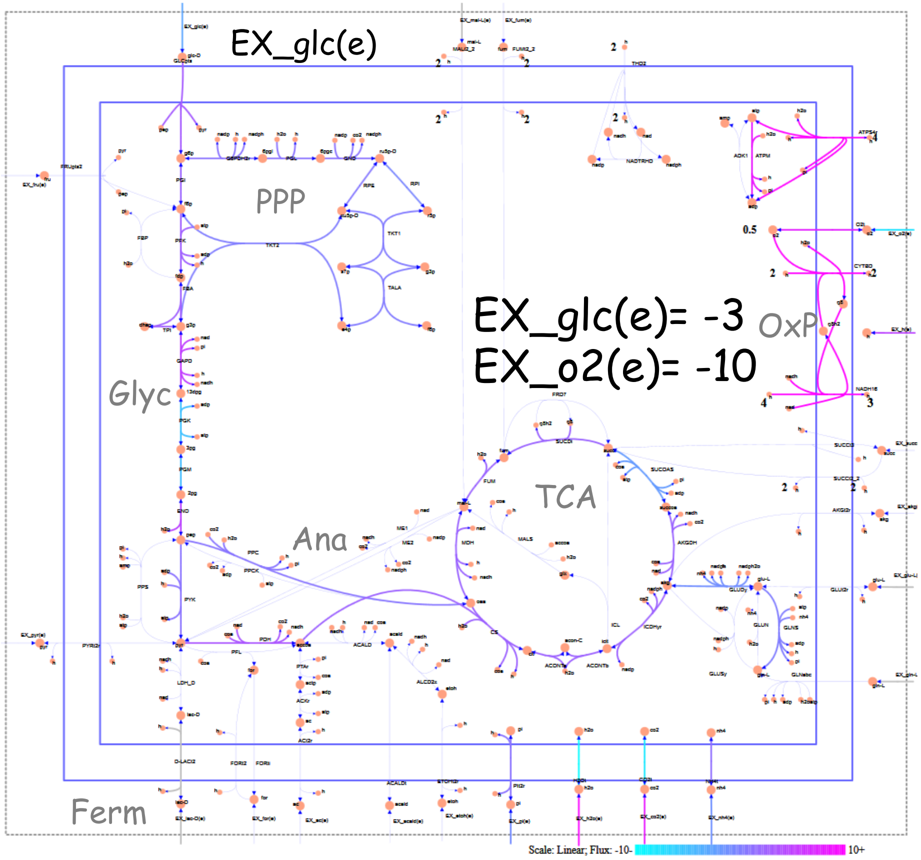
Ratio of Shadow Prices (α > 0)

α > 0

Phases in the PhPP can also have a **positive α value**, termed 'futile' phases. In these phases, one of the substrates is inhibitory toward obtaining the objective function, and this substrate will have a positive shadow price. The metabolic operation in this phase is wasteful, in that it consumes substrate that is not needed to improve the objective. Phases with positive α values are expected to be **phenotypically unstable**, i.e., a cell would not be expected to choose a state in such a region. **Under selection pressure, cells would move their phenotype state out of the phase.**

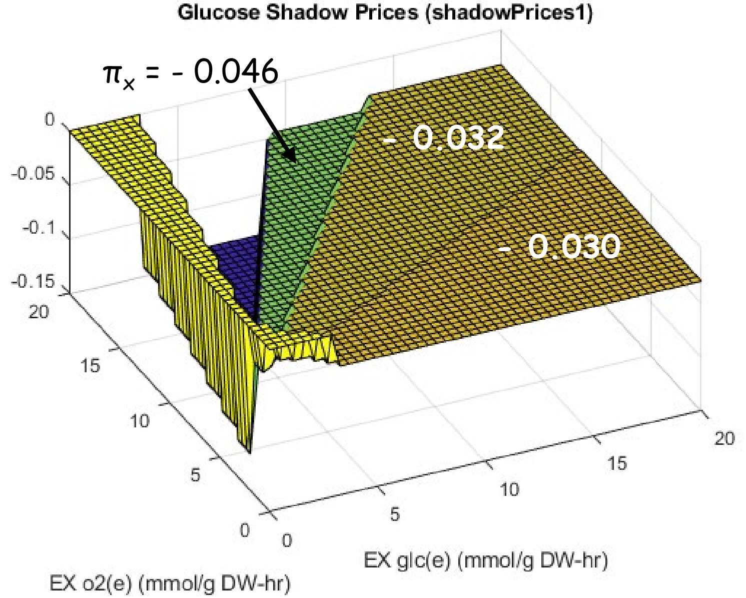


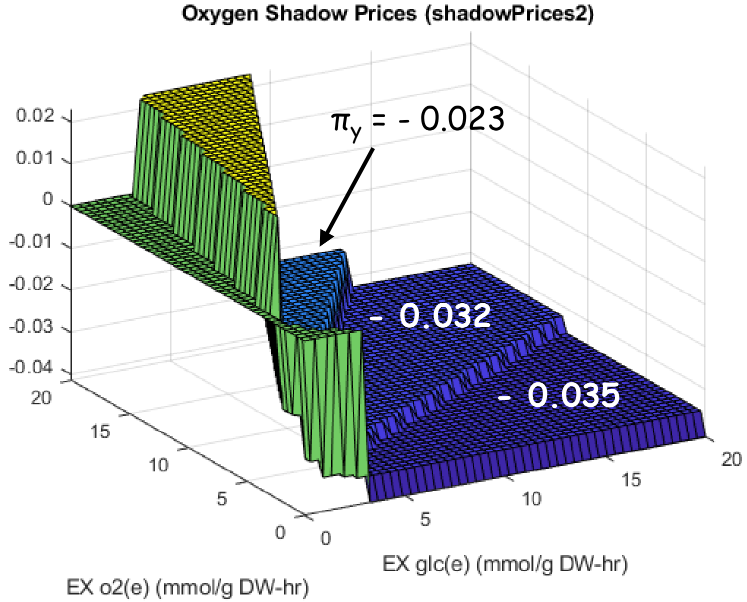
Growth is limited by excess oxygen; not enough glucose to reduce all the oxygen and produce biomass optimally

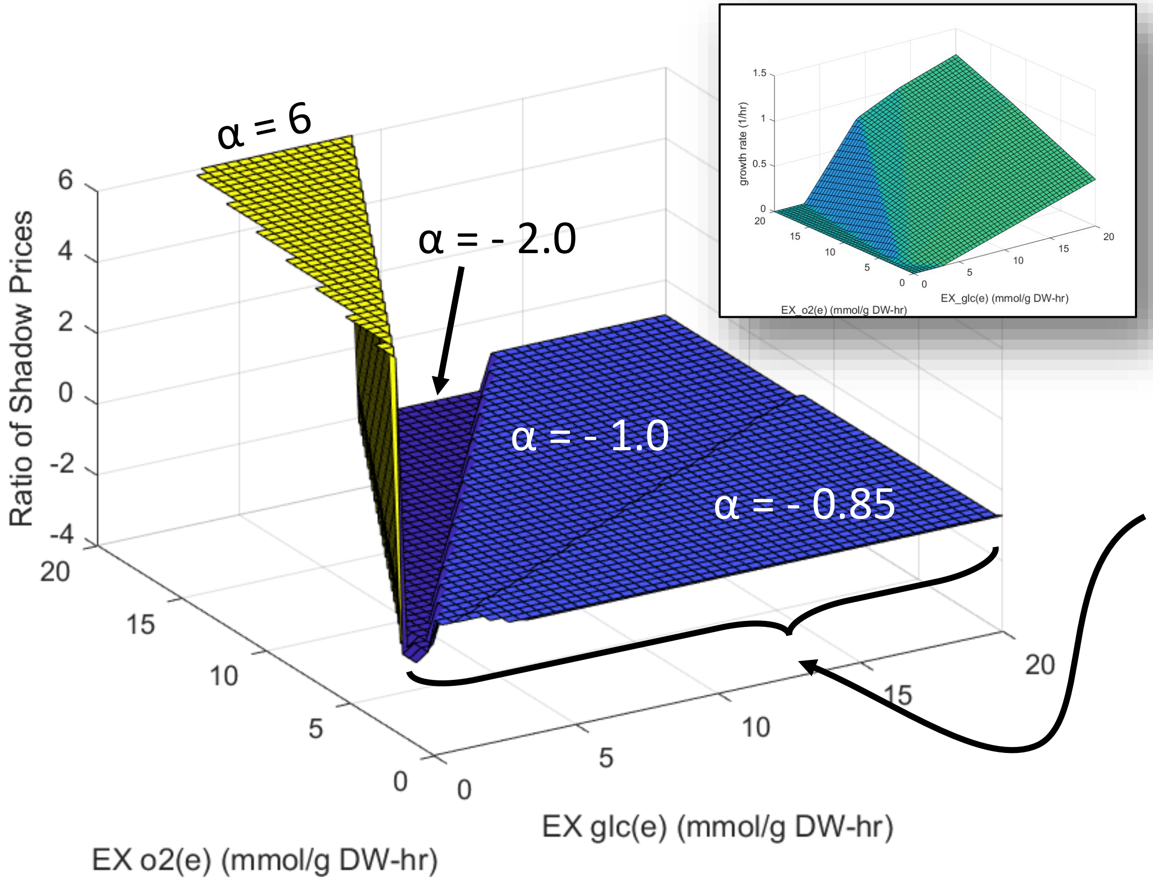


AerobicGlucoseBioMassPhase2.m

Ratio of Shadow Prices (α < 1)





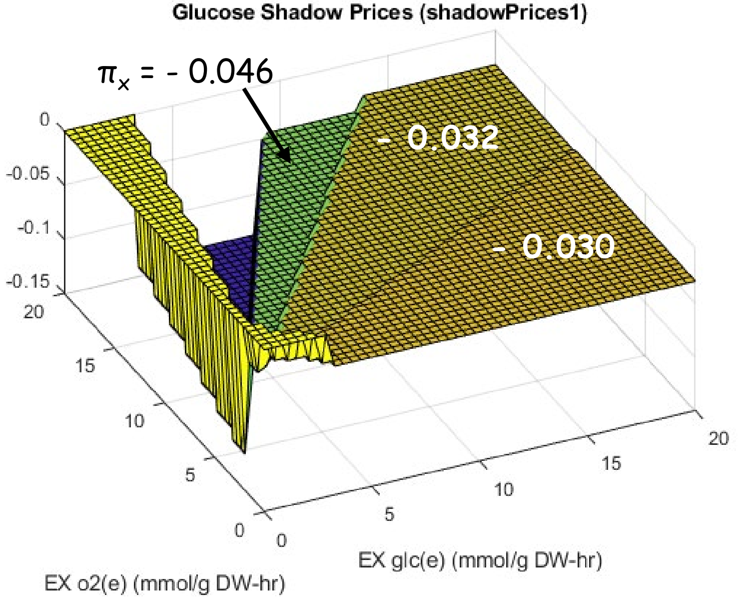


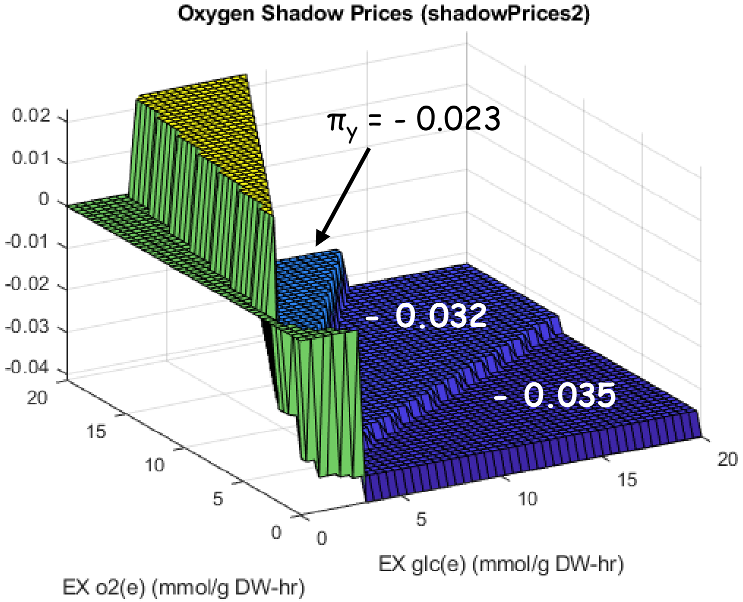
α < 0

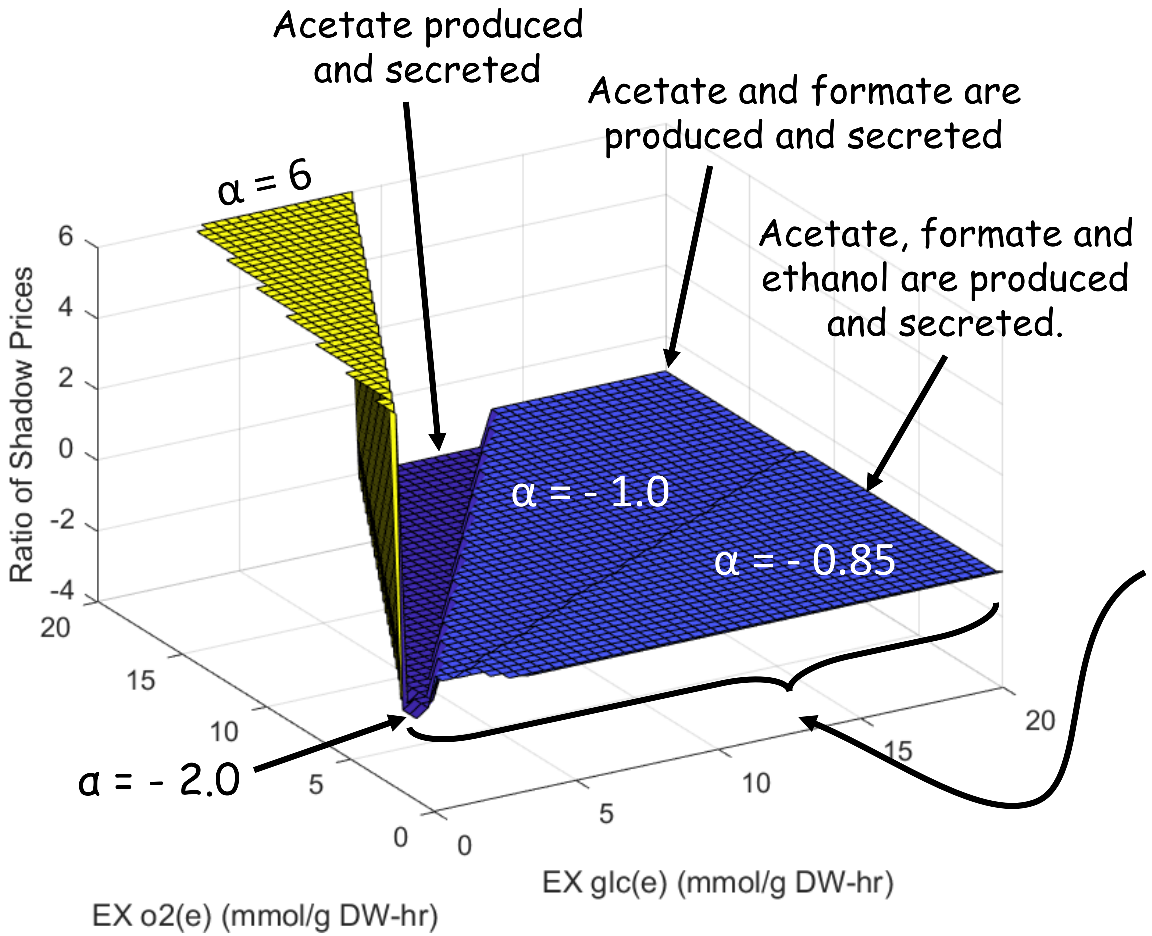
In phases where the **α value is negative**, there is **dual limitation** of the substrates. Based on the absolute value of α, the substrate with a greater contribution toward obtaining the objective can be identified. If the ***absolute value of α is greater than unity, the substrate along the x-axis is more valuable toward obtaining the objective, whereas if the absolute value of α is less than unity, the substrate along the y-axis is more valuable to the objective***.

AerobicGlucoseBioMassPPP_alpha.m

Ratio of Shadow Prices (α < 1)







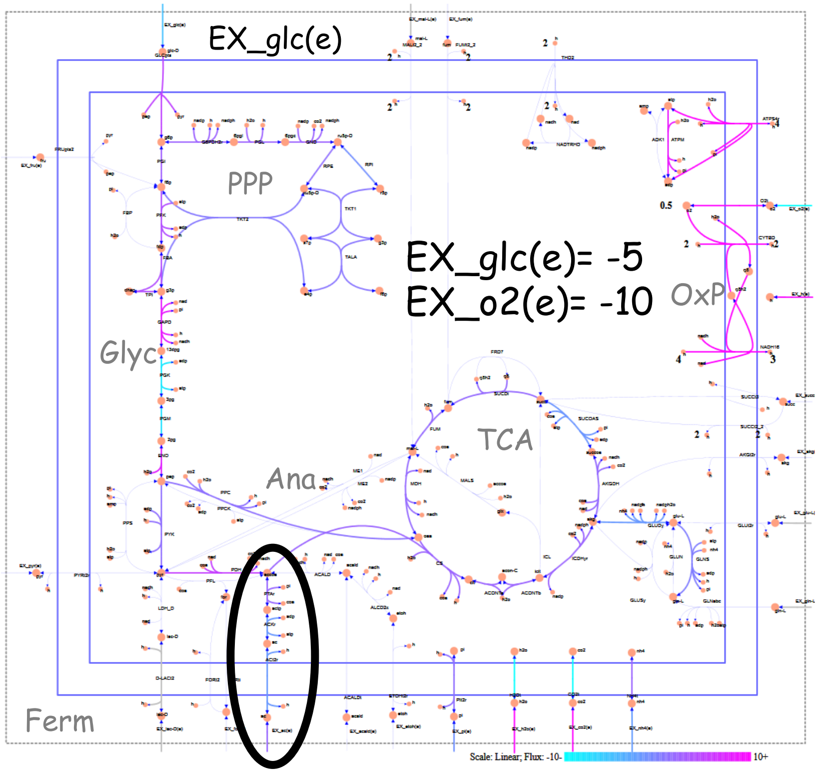
α < 0

In phases where the **α value is negative**, there is **dual limitation** of the substrates. Based on the absolute value of α, the substrate with a greater contribution toward obtaining the objective can be identified. If the ***absolute value of α is greater than unity, the substrate along the x-axis is more valuable toward obtaining the objective, whereas if the absolute value of α is less than unity, the substrate along the y-axis is more valuable to the objective***.

AerobicGlucoseBioMassPPP_alpha.m

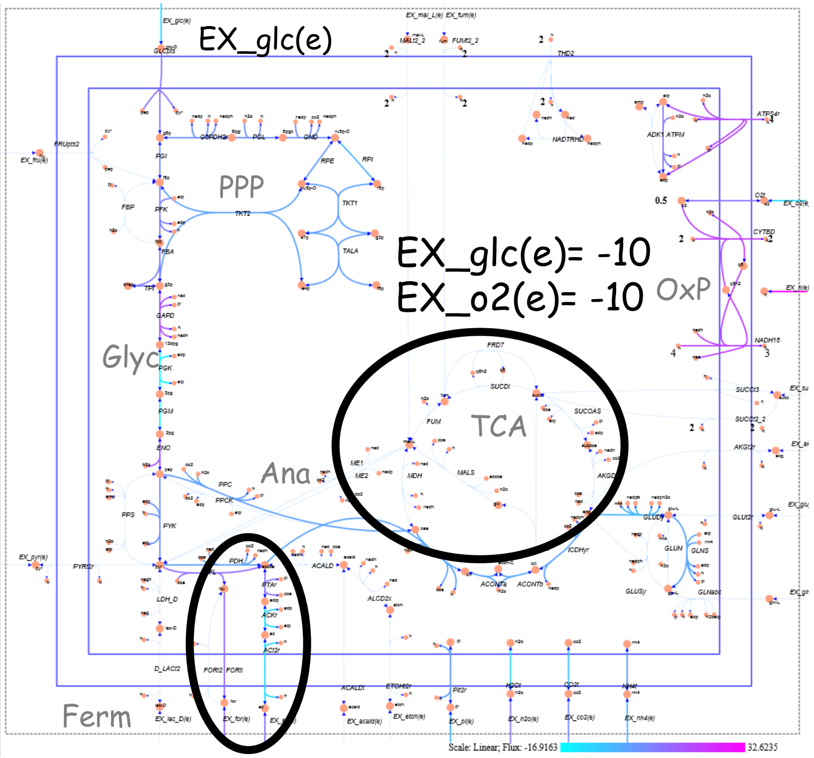
Network Maps: Phase 3-5 (α < 0)

Phase 3 Network Map



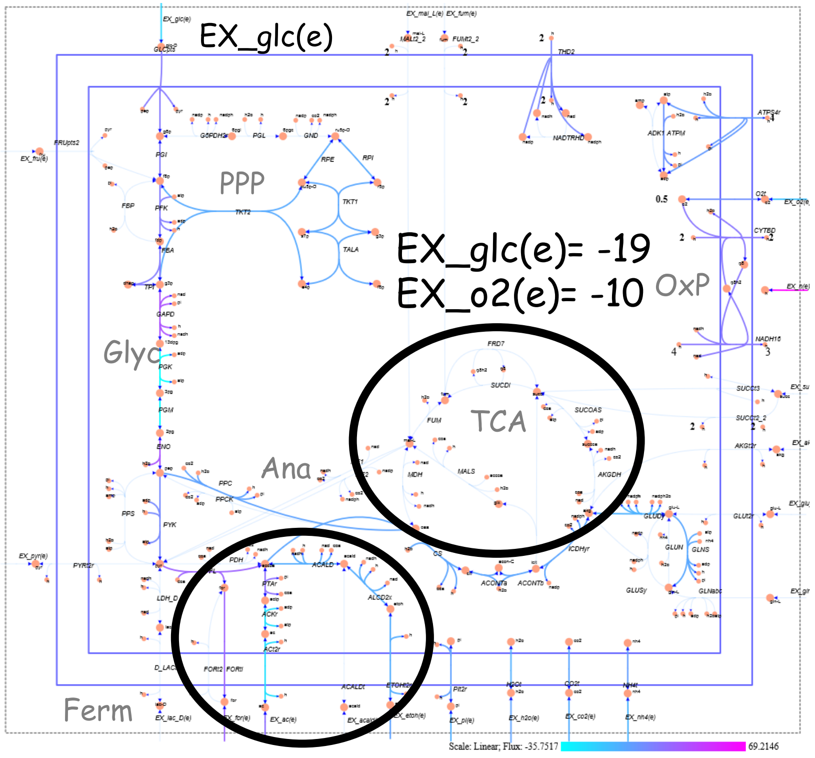
AerobicGlucoseBioMassPhase3.m

Phase 3 Network Map



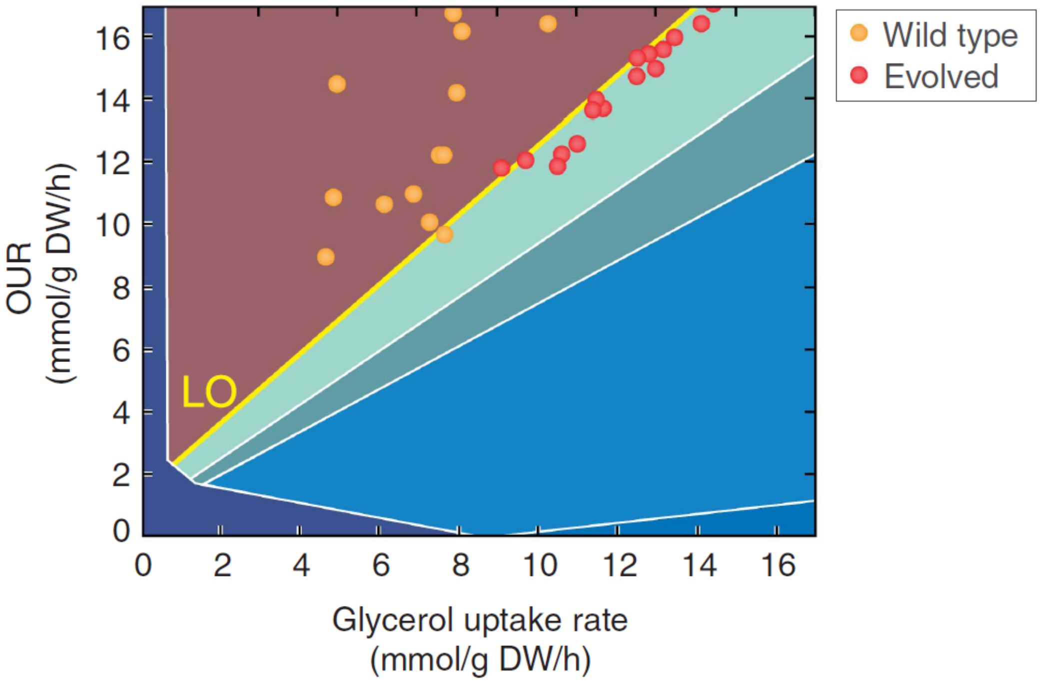
AerobicGlucoseBioMassPhase4.m

Phase 5 Network Map



AerobicGlucoseBioMassPhase5.m

Adaptive Laboratory Evolution



A phenotypic phase plane is a representation of how two fluxes in a metabolic network relate to each other and affect *in silico*-predicted optimal growth. Distinct planes are represented by several colors. Here, the line of optimality (LO, yellow) defines the ratio of glycerol uptake rate to oxygen uptake rate (OUR) that leads to optimal biomass production. On glycerol, wild-type *E. coli* initially has a phenotype that maps to a suboptimal region of the portrait. After a growing for several hundred generations on glycerol, the *E. coli* phenotype migrates to the line of optimality.

Conrad, T. M., N. E. Lewis, et al. (2011). "Microbial laboratory evolution in the era of genome-scale science." Molecular Systems Biology 7: 509.

Lesson Outline

- Robustness Analysis
- Shadow Prices
- Reduced Costs
- Phenotype Phase Plane Analysis

New Cobra Toolbox Functions

1. robustnessAnalysis(model,'EX_glc(e)',100);
    - Shadow Prices located at FBAsolution.y
    - Reduced Costs located at FBAsolution.w
2. printShadowPriceVector(model, - FBAsolution.y, true)
3. phenotypePhasePlane(model, 'rxn1','rxn2')

Reflective Questions

1. What is the purpose of robustness analysis?
2. What do the kinks in the robustness analysis represent?
3. What are shadow prices?
4. How are shadow prices related to the objective function?
5. What are reduced costs?
6. How are reduced costs related to the objective function?
7. What is the difference between shadow prices and reduced costs?
8. What should you use to predict the impact of a metabolite on the objective function, shadow prices or reduced costs?
9. What should you use to predict the impact of a reaction on the objective function, shadow prices or reduced costs
10. What is the purpose of phenotype phase plane analysis?
11. How is robustness analysis related to phenotype phase plane analysis?
12. What is the relationship between shadow prices and phenotype phase plane analysis?
13. What is the purpose of the variable α?
14. What is the line of optimality?
15. How do the different phases created in the phenotype phase plane analysis connect to the physiology of a cell?

Learning Objectives

- Explain the capabilities of robustness analysis
- Explain how shadow prices can be used in metabolic modeling
- Explain how reduced costs can be used in metabolic modeling
- Explain the capabilities of phenotype phase plane analysis

References

Robustness Analysis

- Edwards, J. S. and B. O. Palsson (2000). "Robustness analysis of the Escherichia coli metabolic network." Biotechnology progress 16(6): 927-939.
- Orth, J. D., I. Thiele, et al. (2010). "What is flux balance analysis?" Nature biotechnology 28(3): 245-248. (Supplementary Tutorial)
- Price, N. D., J. A. Papin, et al. (2003). "Genome-scale microbial in silico models: the constraints-based approach." Trends in biotechnology 21(4): 162-169

Phenotype PhasePlane Analysis

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