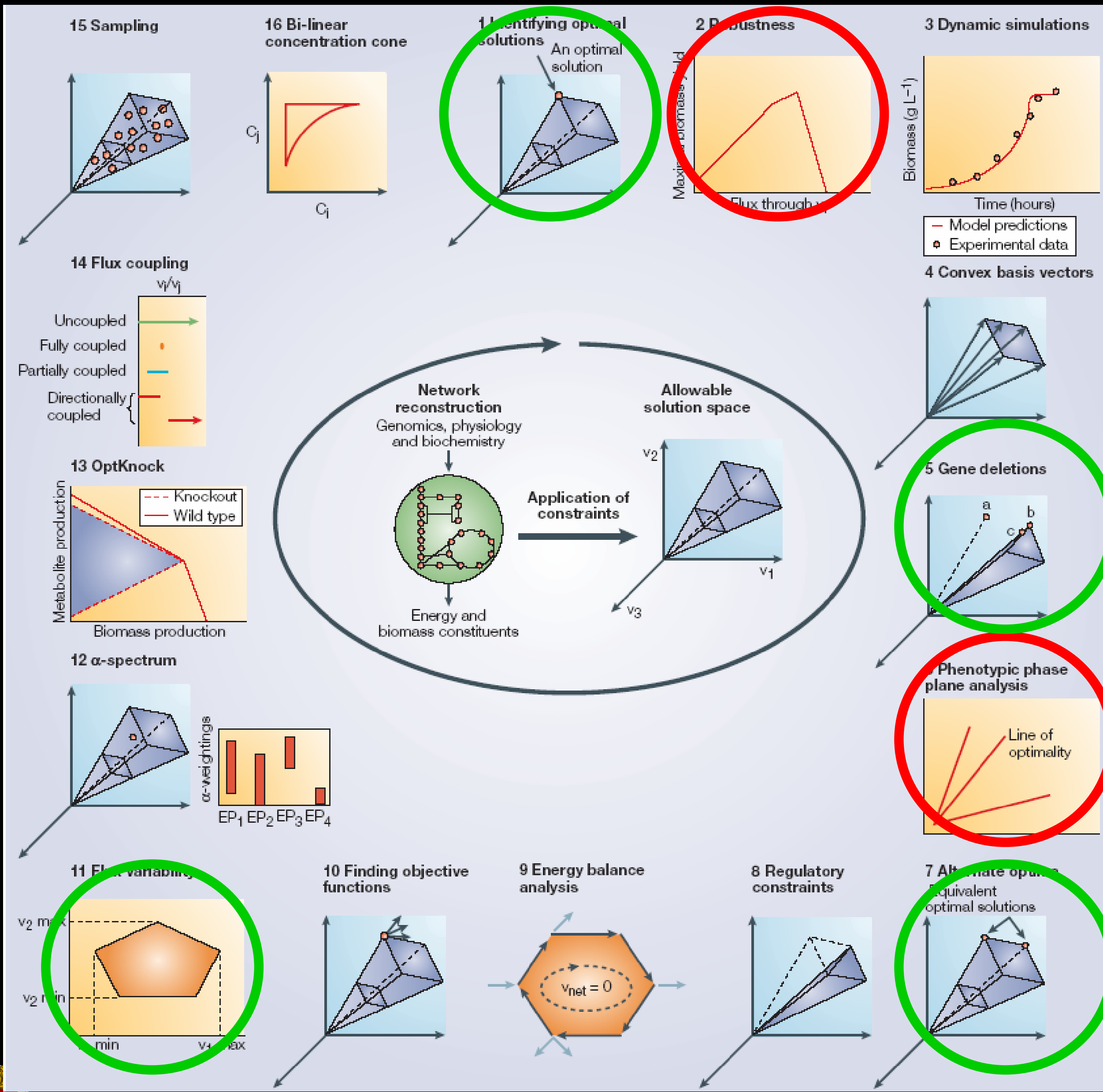
Constraint-Based Workshops

7. Robustness & Phase Planes
February 6th, 2008

No Meeting Next Week!!

Constraint-Based Methods



Optimal Solutions
1. FBA
2. Flux Variability

Flux Dependencies
1. Robustness
2. Phase Planes
3. Flux Coupling

All Allowable Solutions
1. Extreme Pathways
2. Elementary Modes
3. Sampling

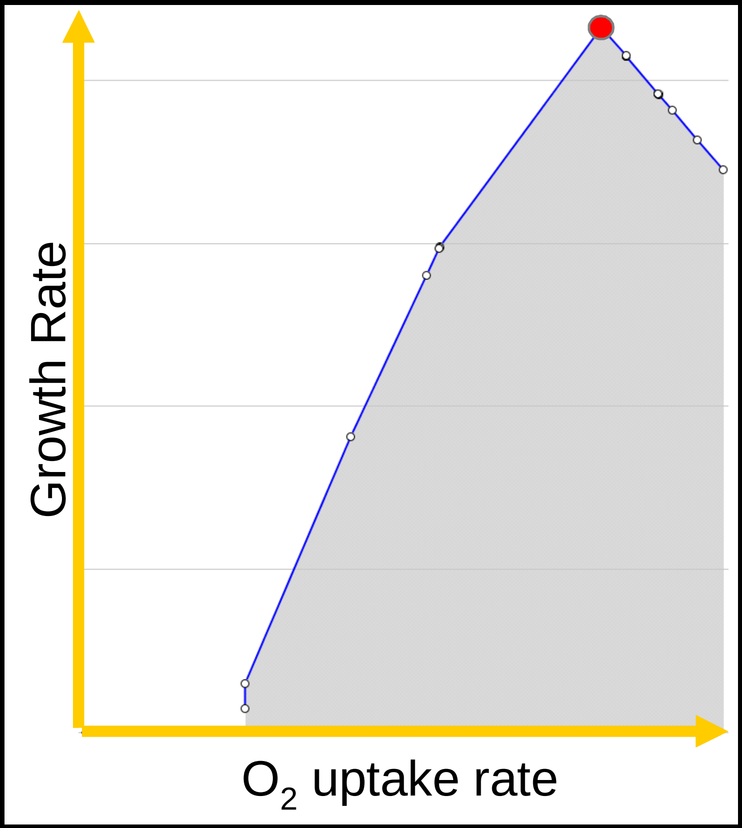
Altering Phenotypes
1. Genetic Mutations
2. Strain Design

Application of Additional Constraints
1. Regulation
2. Energy Balance

Price, Reed, and Palsson
Nat. Reviews Microbiol.
2004

Robustness Analysis

Used to calculate how the objective function changes to incremental changes in a particular flux.



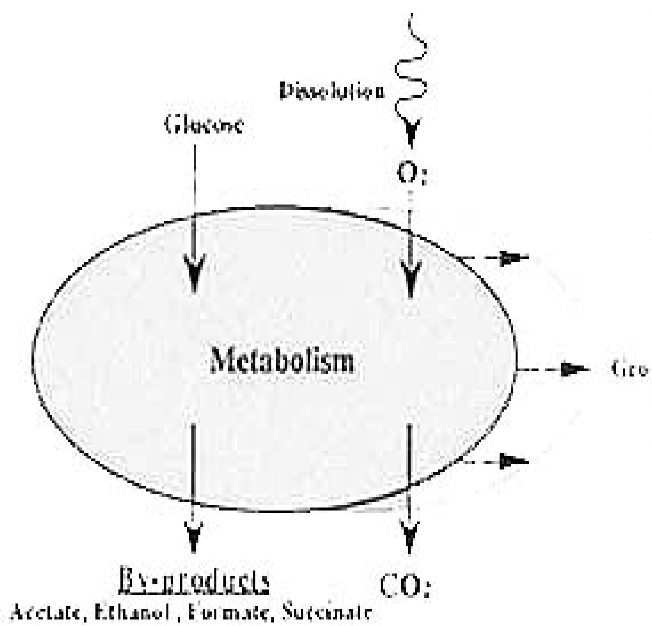
Curves are piecewise linear w/slope equal to Shadow Price

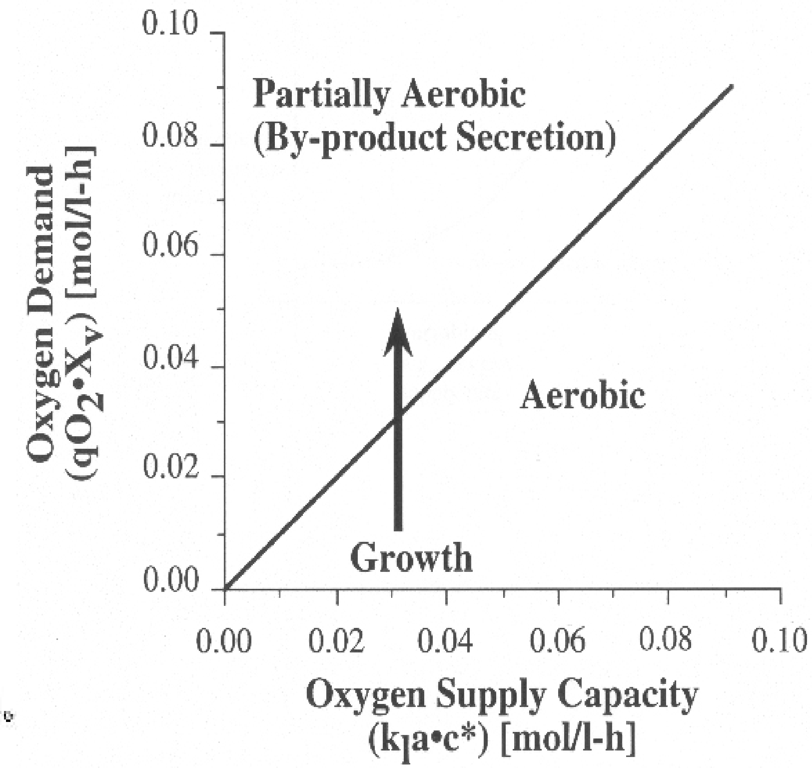
Oxygen Limitations and By-product Secretion

Restriction to a finite capacity

Uptake Limits

- Enzymatic limits
- Mass Transfer limits
- Supply Restrictions





20 mmol/g DW-hr

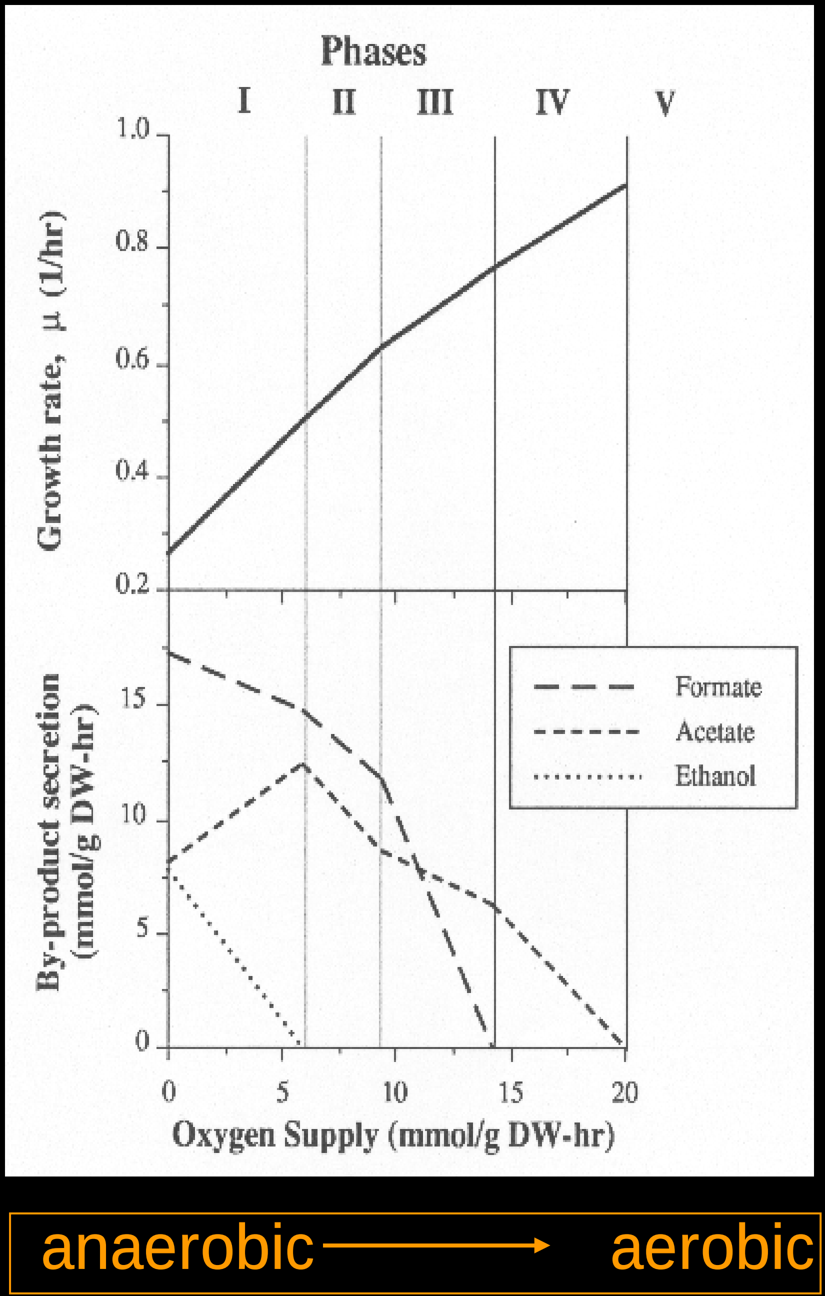
(Respiratory chain limitation)

Andersen and Meyenburg (1980)
J. Bacteriol 144: 114-23

Example

In this example we vary the maximum allowable uptake rate of oxygen. The whole range of oxygenation is shown, from fully aerobic conditions to fully anaerobic conditions.

The growth rate is graphed in the upper panel and the by-product secretion rates in the lower.



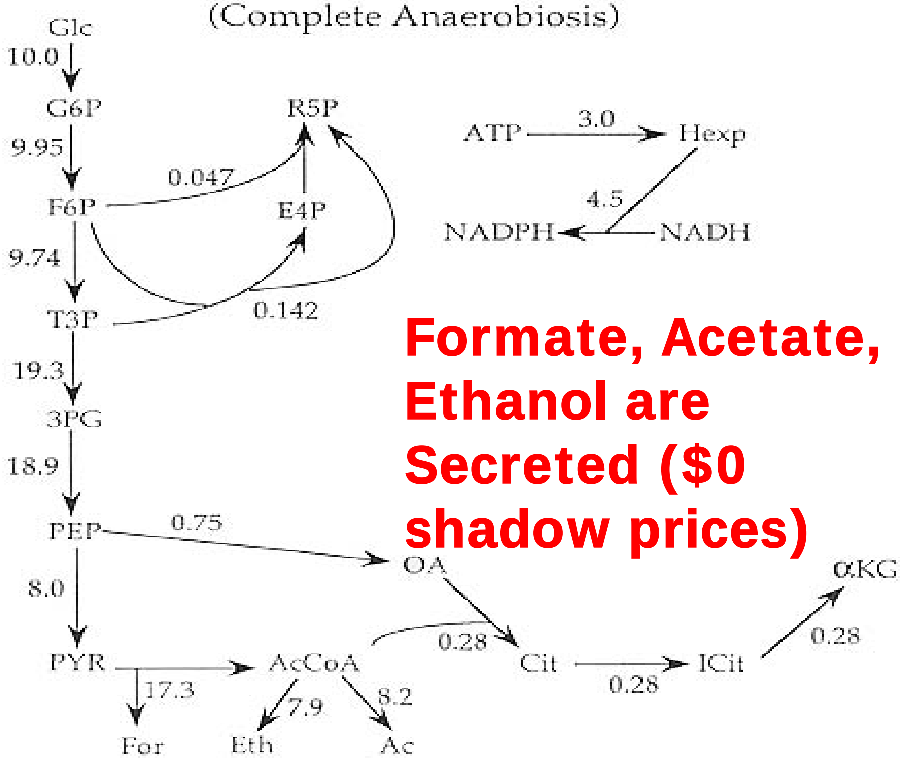
Review of Shadow Prices & Reduced Costs

- Shadow Prices (SP):
  - One for each constraint or metabolite
  - dZ/db_i
  - SP<0 means adding metabolite (ie. change b=0 to b<0) would increase Z.
  - SP>0 means removing metabolite (ie. change b=0 to b>0) would increase Z.
- Reduced Costs (RC):
  - One for each variable or flux.
  - dZ/dv_j (for zero fluxes)
  - RC < 0 means increasing flux (v_j) would reduce Z.

Shadow prices: Interpret changes in optimal solutions

PHASE I

(Complete Anaerobiosis)

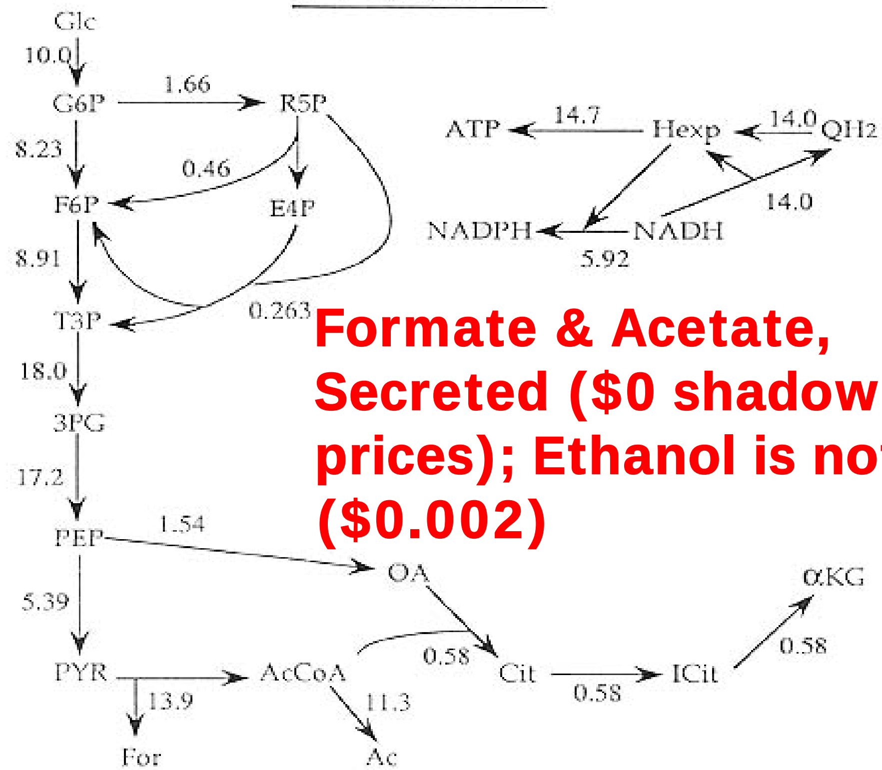


Formate, Acetate, Ethanol are Secreted ($0 shadow prices)

Shadow Prices

O_2	0.040	NADPH	0.0018	Acetate	0.0
		NADH	- 0.0054	Ethanol	0.0
ATP	0.011	FADH	-0.013	Formate	0.0
Hexp	0.0036	QH_2	-0.013	Lactate	0.0054
				Succinate	0.011

PHASE II

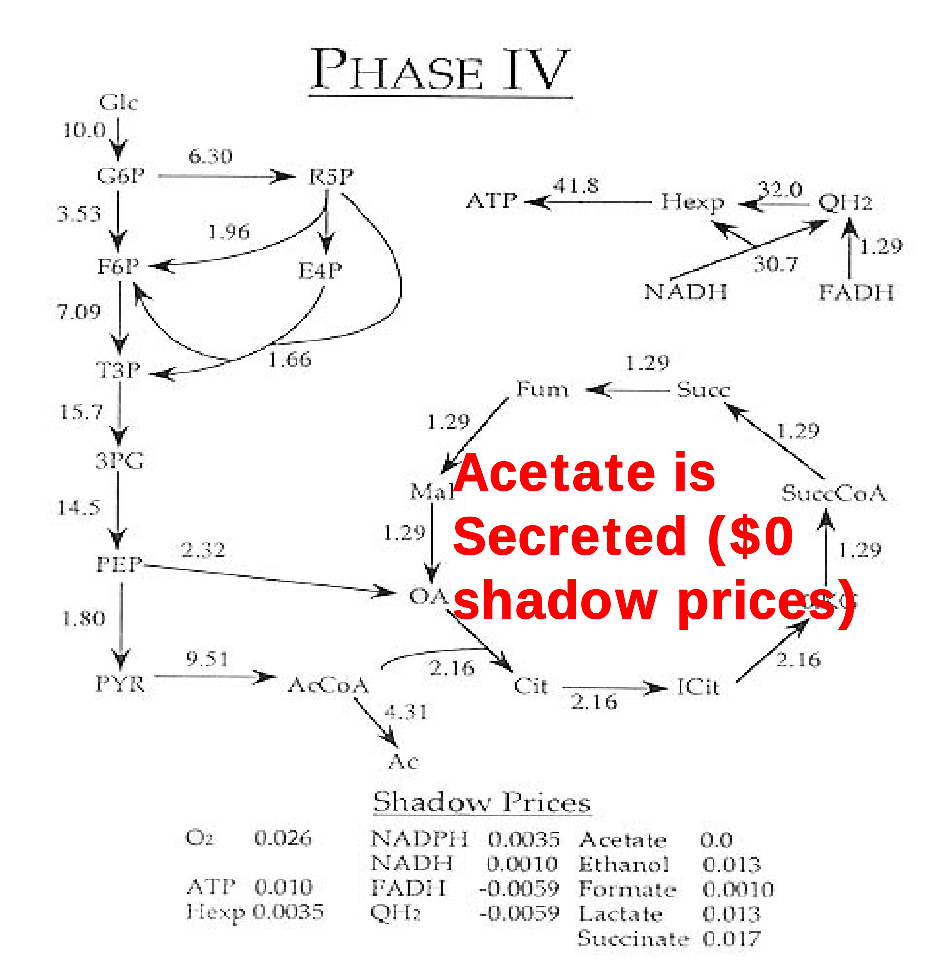


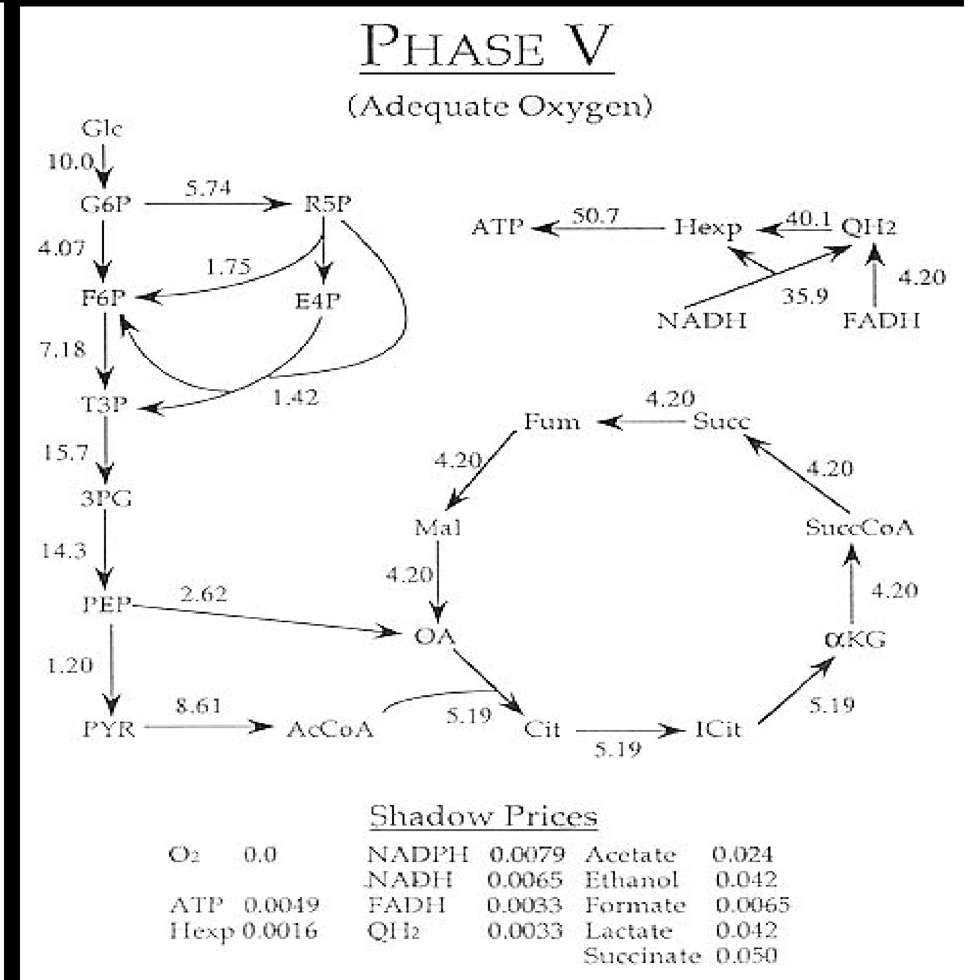
Formate & Acetate, Secreted ($0 shadow prices); Ethanol is not ($0.002)

Shadow Prices

O_2	0.038	NADPH	0.0029	Acetate	0.0
		NADH	- 0.0043	Ethanol	0.0021
ATP	0.011	FADH	-0.011	Formate	0.0
Hexp	0.0036	QH_2	-0.011	Lactate	0.0064
				Succinate	0.013

Flux distributions for different levels (or phases) of oxygenation





partially anaerobic ⟶ aerobic

Robustness Analysis

coretextbookmodel.gms | robustness.gms

```
**THIS CODE WAS WRITTEN FOR SYSTEMS BIOLOGY SHORT COURSE BY J.REED (7/2005)

*Read in the appropriate S matrix
$include CoreTextbookModel.gms

*Place limits on the exchange fluxes based on the minimal media
*for a negative flux through the exchange reactions implies that
*the metabolites are being taken up or consumed by the cell.
*By default the upperlimits of the exchange fluxes are all set to
*the Vmax, indicating that the cell can secrete any of the extracellular
*metabolites
UpperLimits(j)=Vmax;
*CARBON SOURCE: select upper and lower limits for exchange flux
LowerLimits('EX_glc_e')=-1;
UpperLimits('EX_glc_e')=-1;
*allow co2,pi,o2,h,h2o to be taken up by the cell
LowerLimits('EX_co2_e')=-Vmax;
LowerLimits('EX_h2o_e')=-Vmax;
LowerLimits('EX_h_e')=-Vmax;
LowerLimits('EX_o2_e')=-Vmax;
LowerLimits('EX_pi_e')=-Vmax;

*Define the number of steps that you want to take eg. /step1*step25/ will have 25 steps
steps /step1*step25/;

Parameter
c(j)    used to define the objective function for optimization
n_steps  number of steps that will be taken and is defined by the elements in steps
range_max maximum flux value through the flux to be varied
range_min minimum flux value through the flux to be varied
flux_value(steps)  stores the values for the varied flux
store_obj(steps) stores the value of the objective function for each iteration
pick_flux(j) a vector of zeros except for the one flux which will be varying;

*Determine the number of steps based on the number of elements in the set steps
n_steps=card(steps);
*Select the flux that you want to vary
pick_flux('TKT1')=1;
```

Define Number of Steps

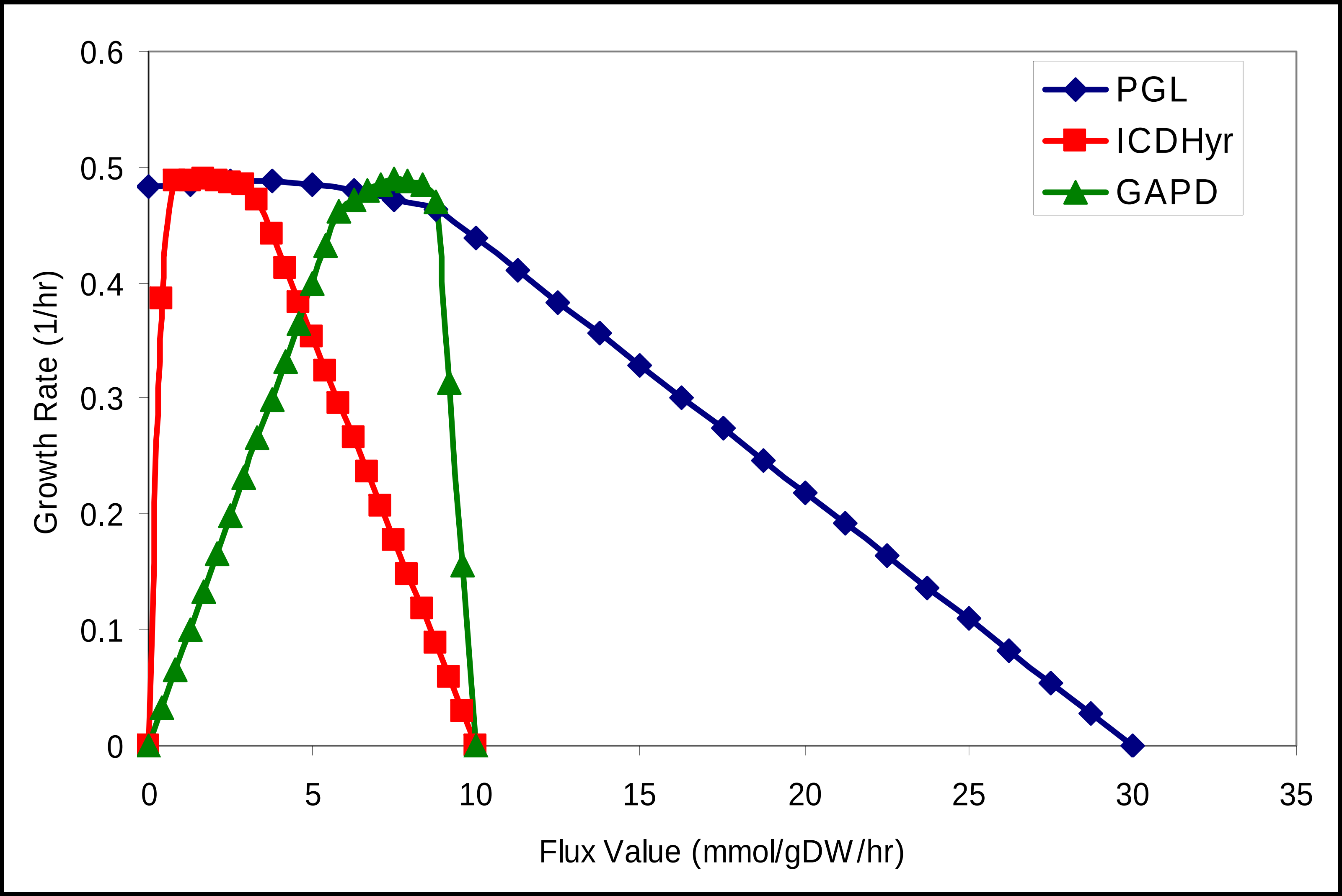
Define Flux to Vary

Robustness Analysis Calculations

- Calculate the sensitivity of the objective function to changes in, *use glucose uptake rate of 5 and aerobic conditions*:
  - PGL (pentose phosphate flux)
  - GAPD (glycolytic flux)
  - ICDHyr (TCA cycle flux)

Graph results in excel!

Robustness Analysis



What Does this Mean?

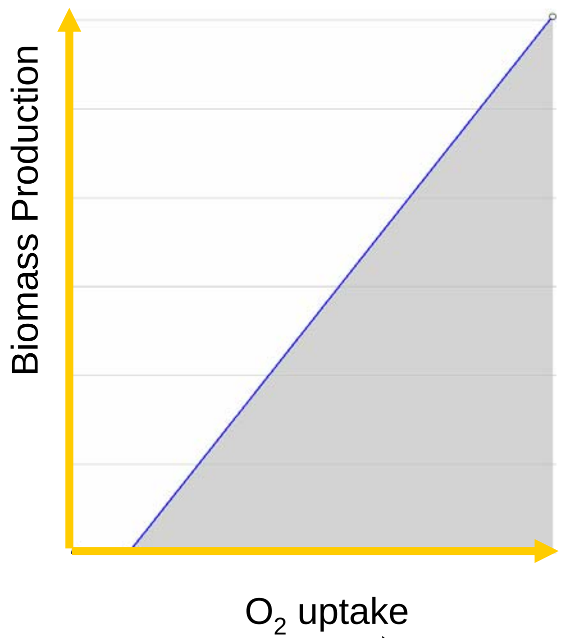
- Which reaction(s) are essential (note that FBA, MOMA, and ROOM would all predict the same lethal phenotype)?

Phase Plane Analysis:

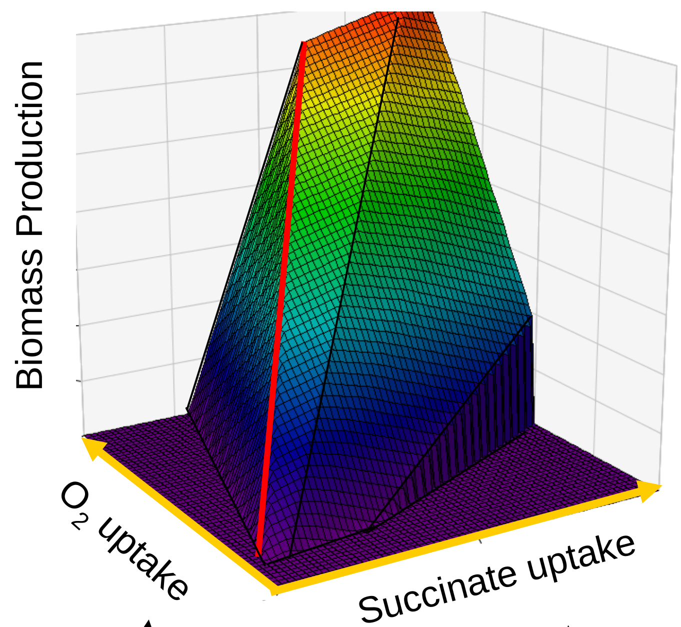
Varying multiple fluxes
simultaneously

Parameter Variation

Robustness Analysis: Projection of PhPP for Maximum Growth rate vs. O_2 uptake

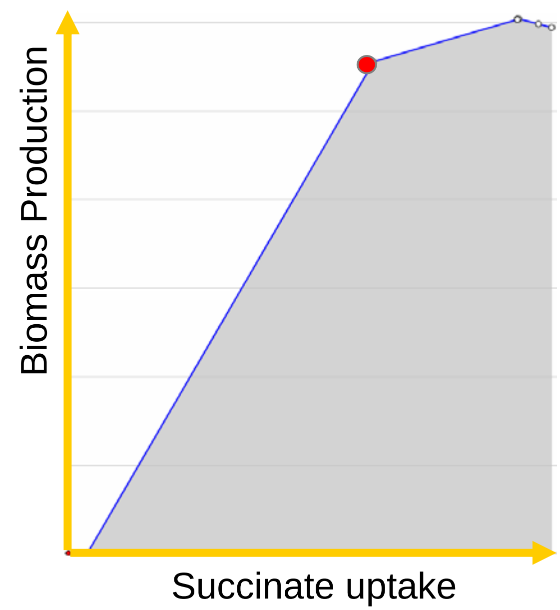


Phenotypic Phase Plane (PhPP)



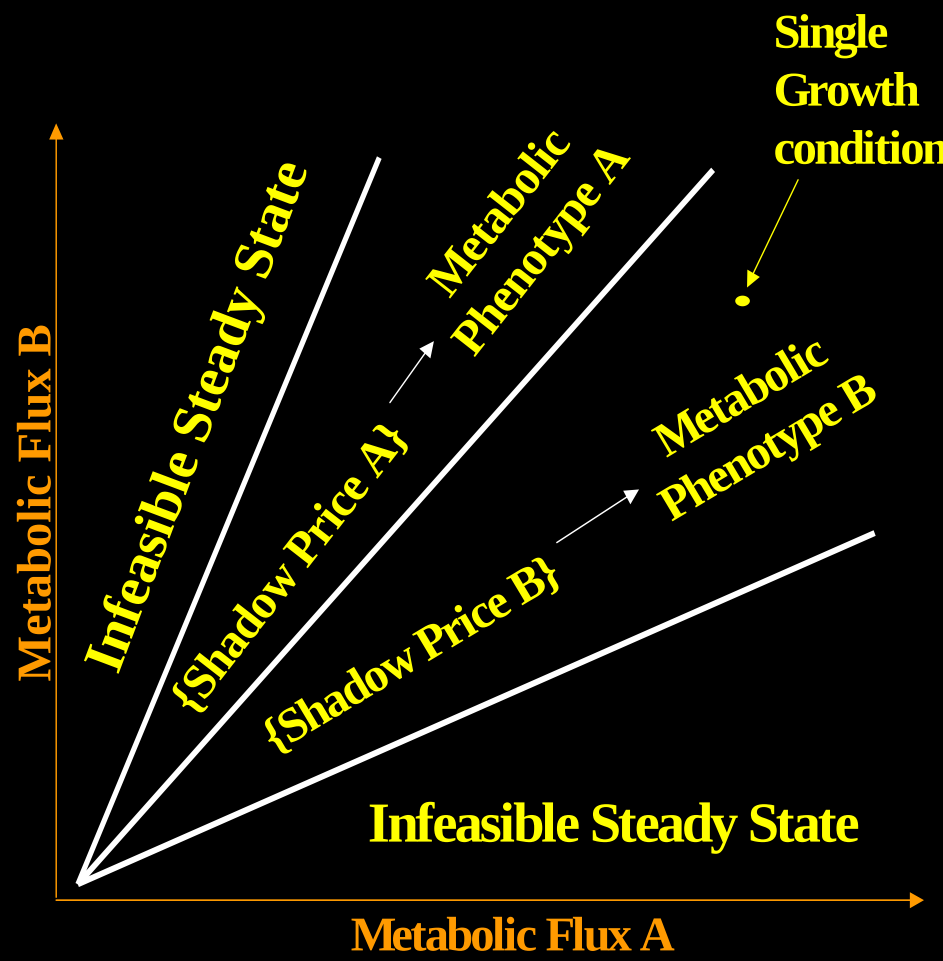
Line of Optimality (LO)

Robustness Analysis: Projection of PhPP for Maximum Growth rate vs. Succinate uptake



Phenotype Phase Plane

- 2-dimensional region
  - Spanned by 2 metabolic fluxes
    - Typically uptake rates
  - lines to demarcate phase of constant shadow price
  - By definition, metabolic pathway utilization is different in each region of the phase plane



Shadow Prices and Isoclines

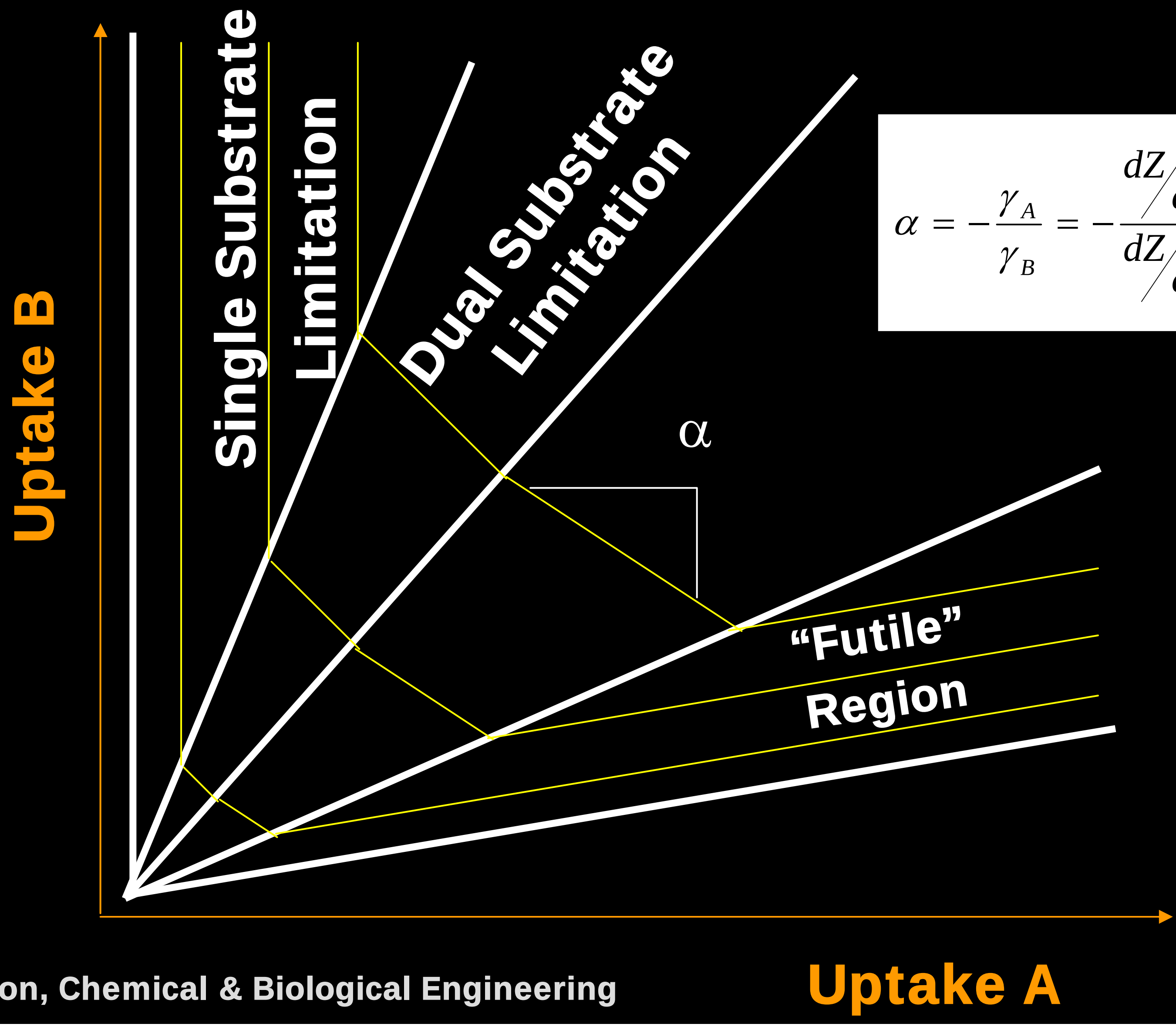
Shadow Price

$$\gamma_i = \left.\frac{\partial Z}{\partial b_i}\right]_{boundary}$$

Relative shadow prices

$$\alpha = -\frac{\gamma_A}{\gamma_B} = -\frac{dZ/db_A}{dZ/db_B} = -\frac{db_B}{db_A}$$

Isoclines:
Lines w/ Constant Objective Values

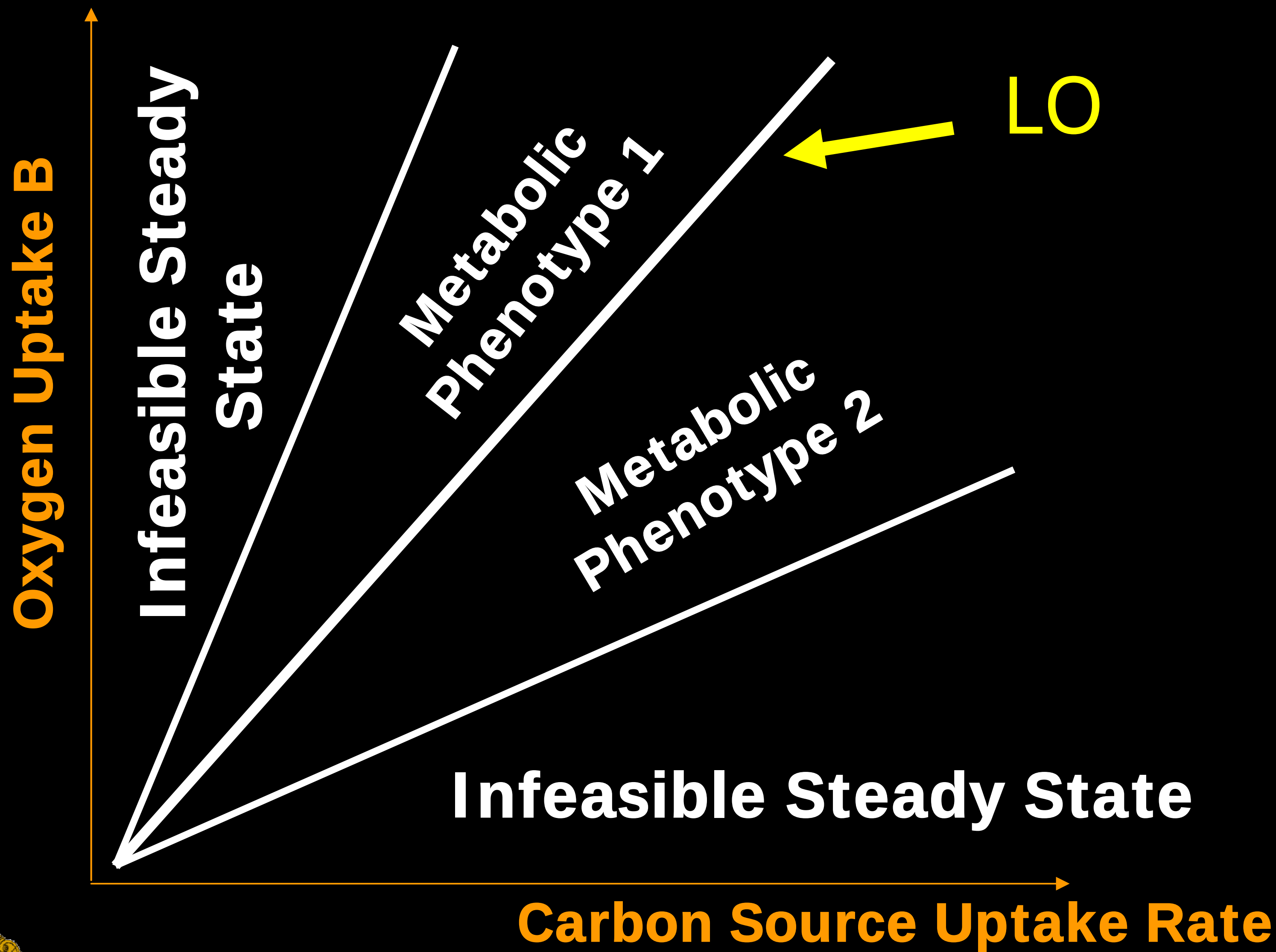


$$\alpha = -\frac{\gamma_A}{\gamma_B} = -\frac{dZ/db_A}{dZ/db_B} = -\frac{db_B}{db_A}$$

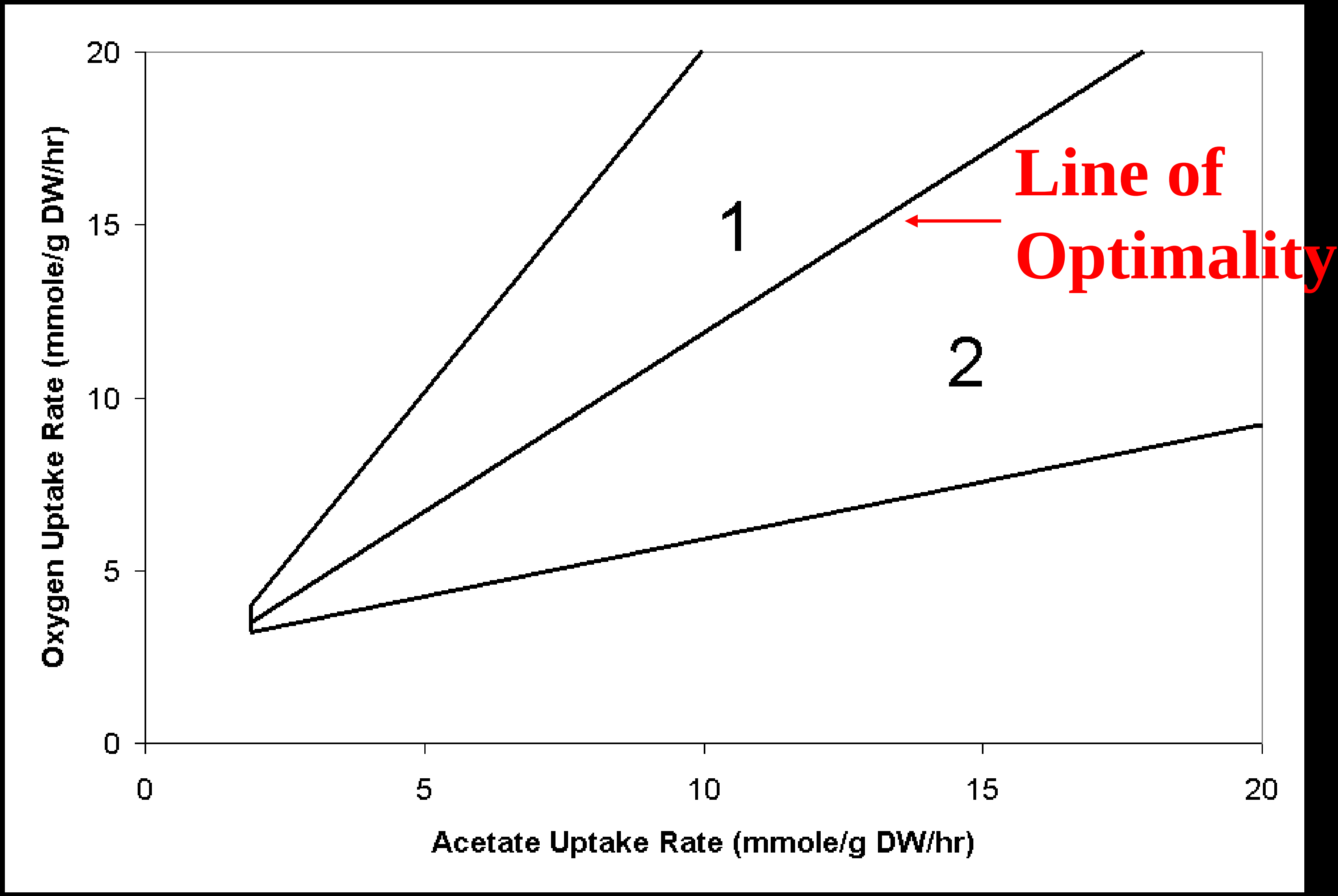
Characteristics of Phase Planes

- Infeasible regions: fluxes don't balance
- Regions of single substrate limitations (α = 0 or infinity)
- Regions of dual substrate limitations (α < 0)
- Futile regions (α >0)
- Isoclines (like constant height in topography maps)
- Line of optimality: corresponds to maximal biomass yield (g cells/mmol carbon source)
  - You find this by fixing carbon uptake rate and the optimize for biomass using FBA, this will give you one point on the LO

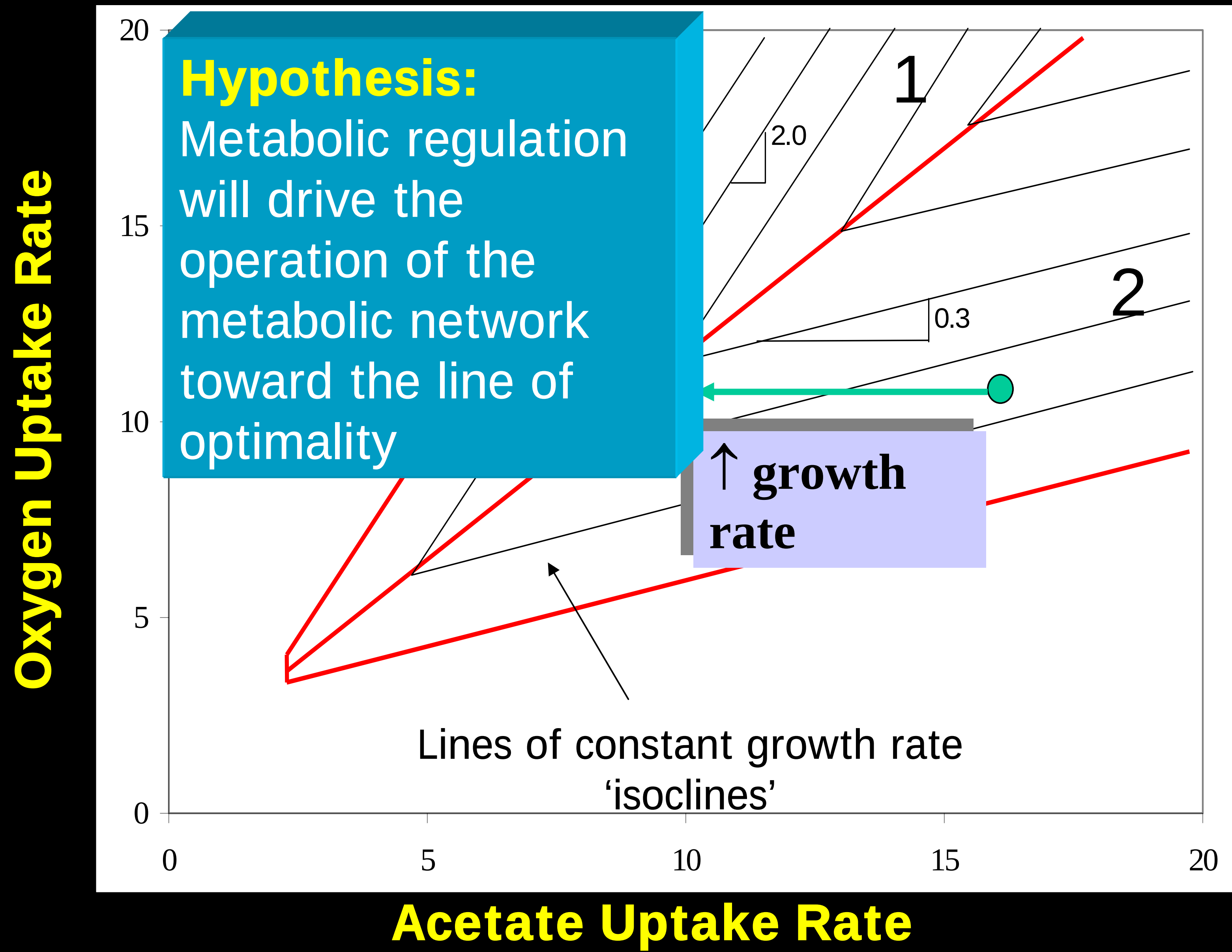
Line of Optimality: Max. $Y_{x/s}$



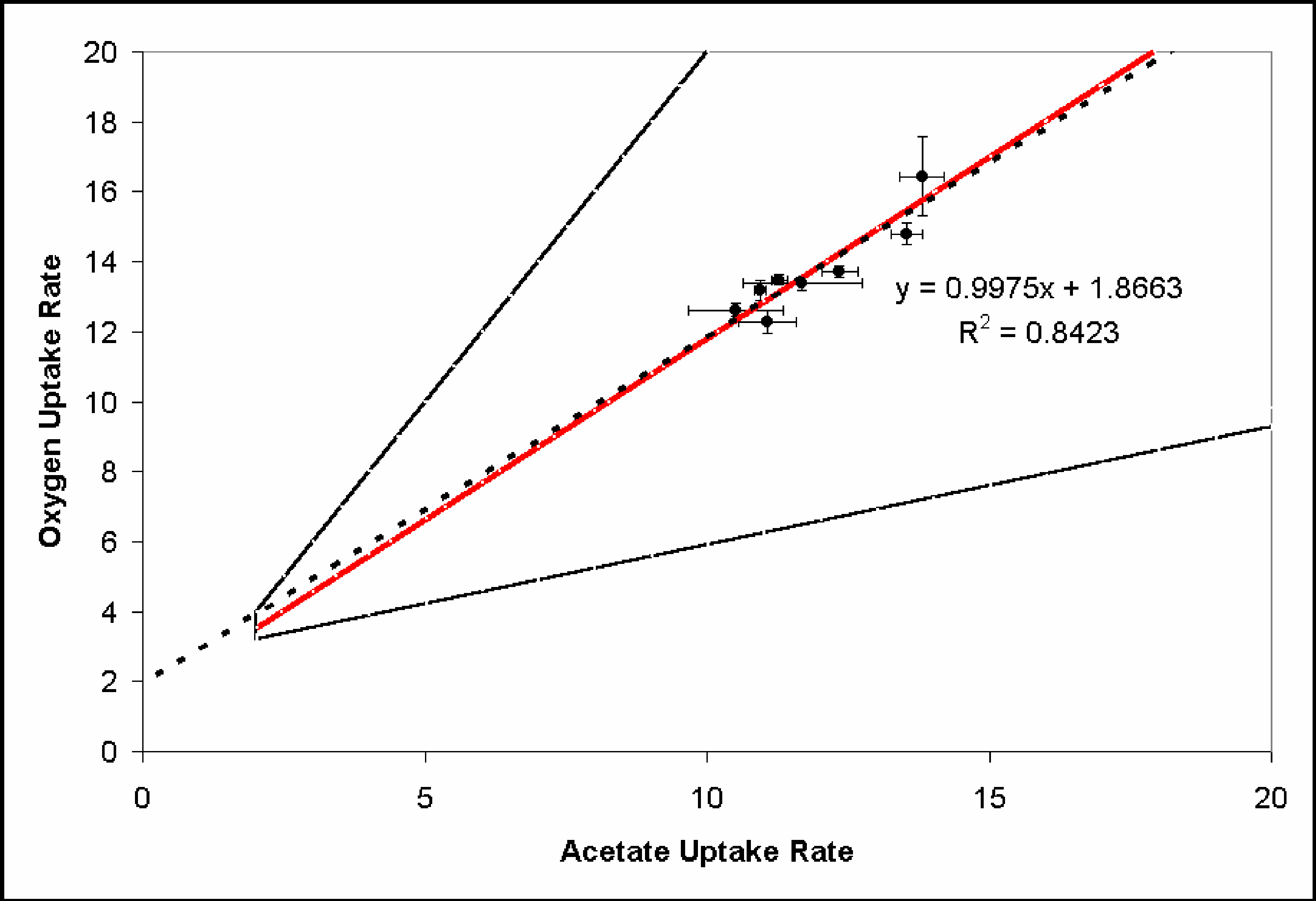
Acetate Phase Plane for E. coli



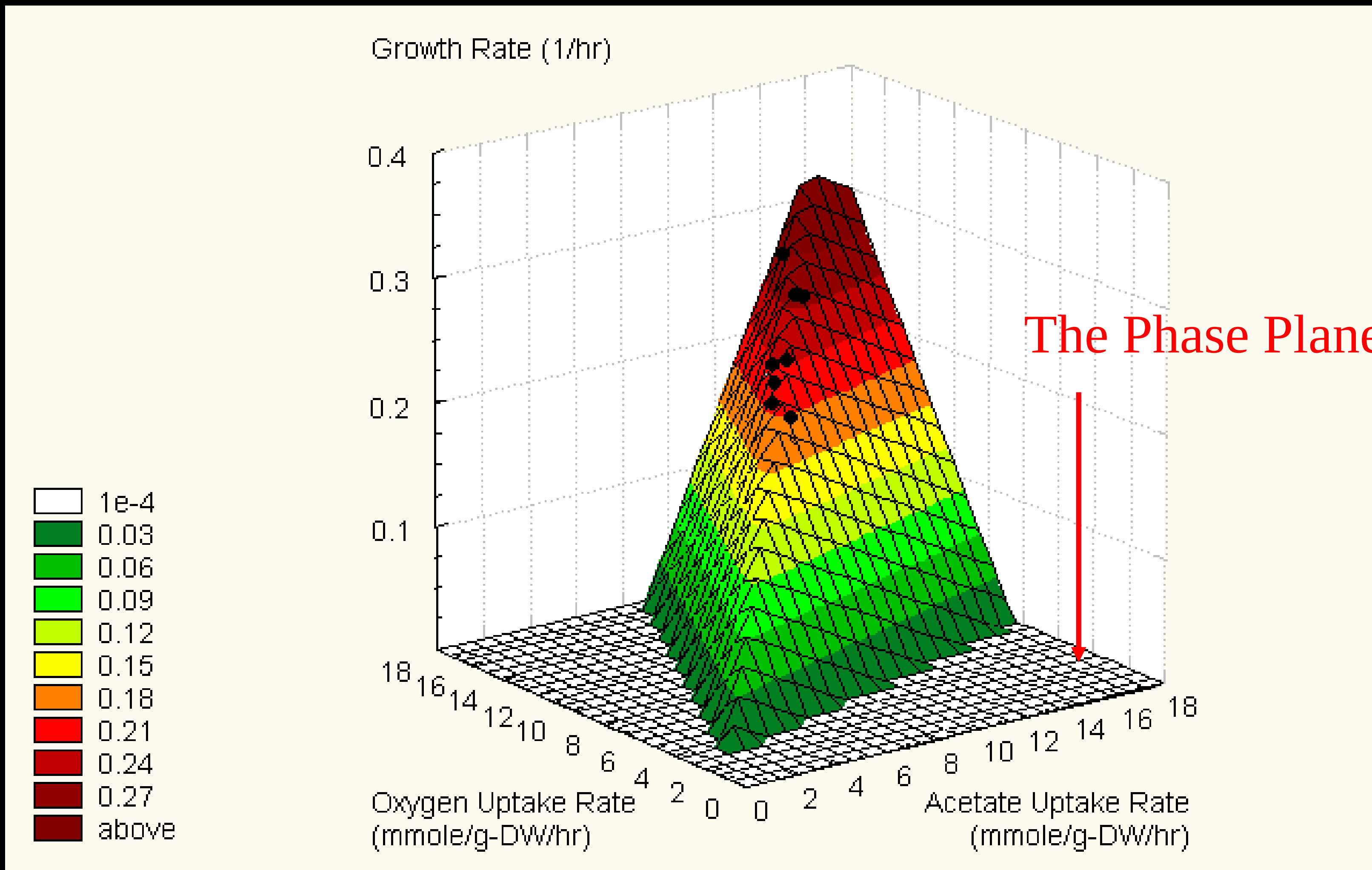
Acetate PhPP: Two Futile Regions



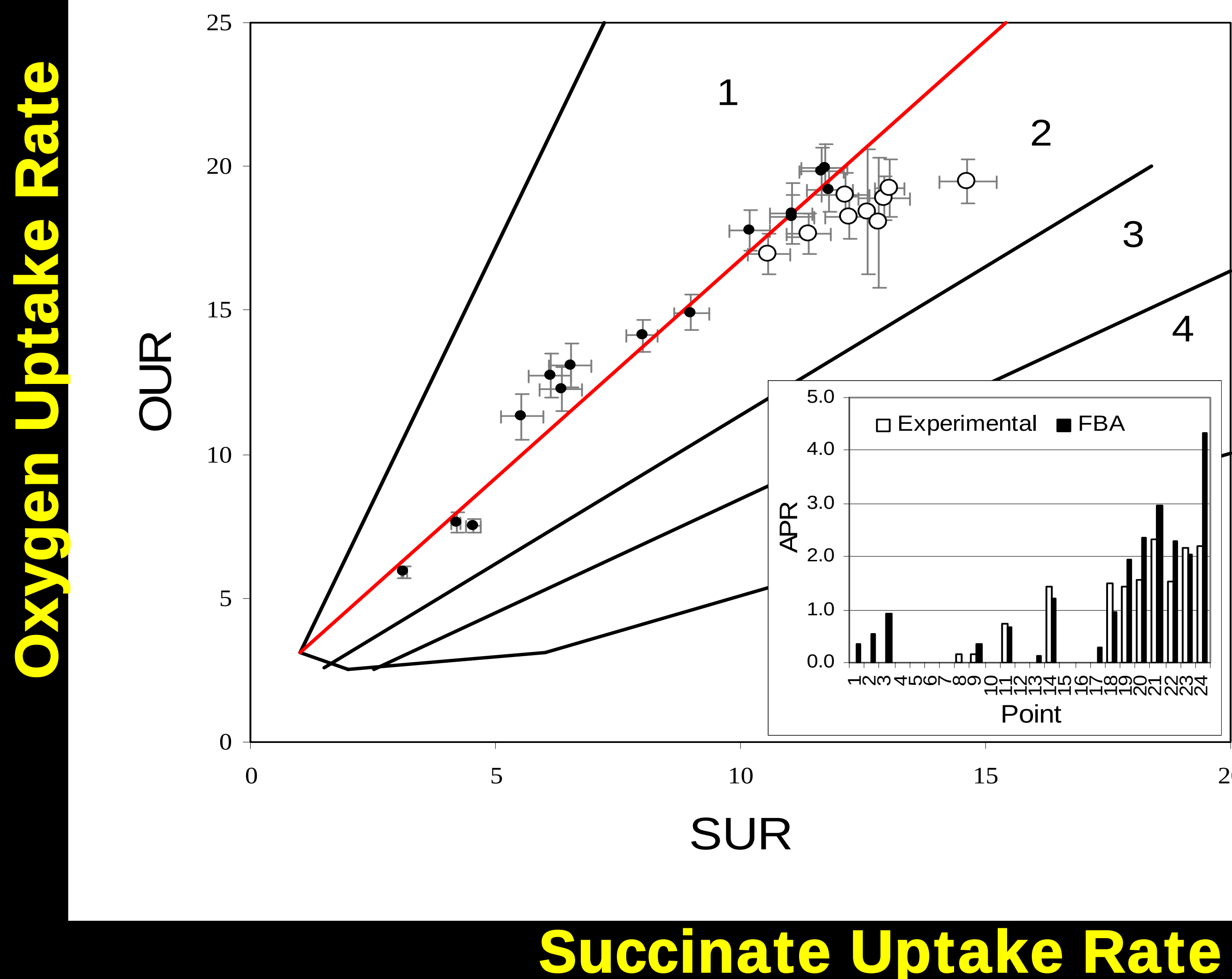
Acetate PhPP & Experimental Data



Growth on Acetate 3D Phase Plane:



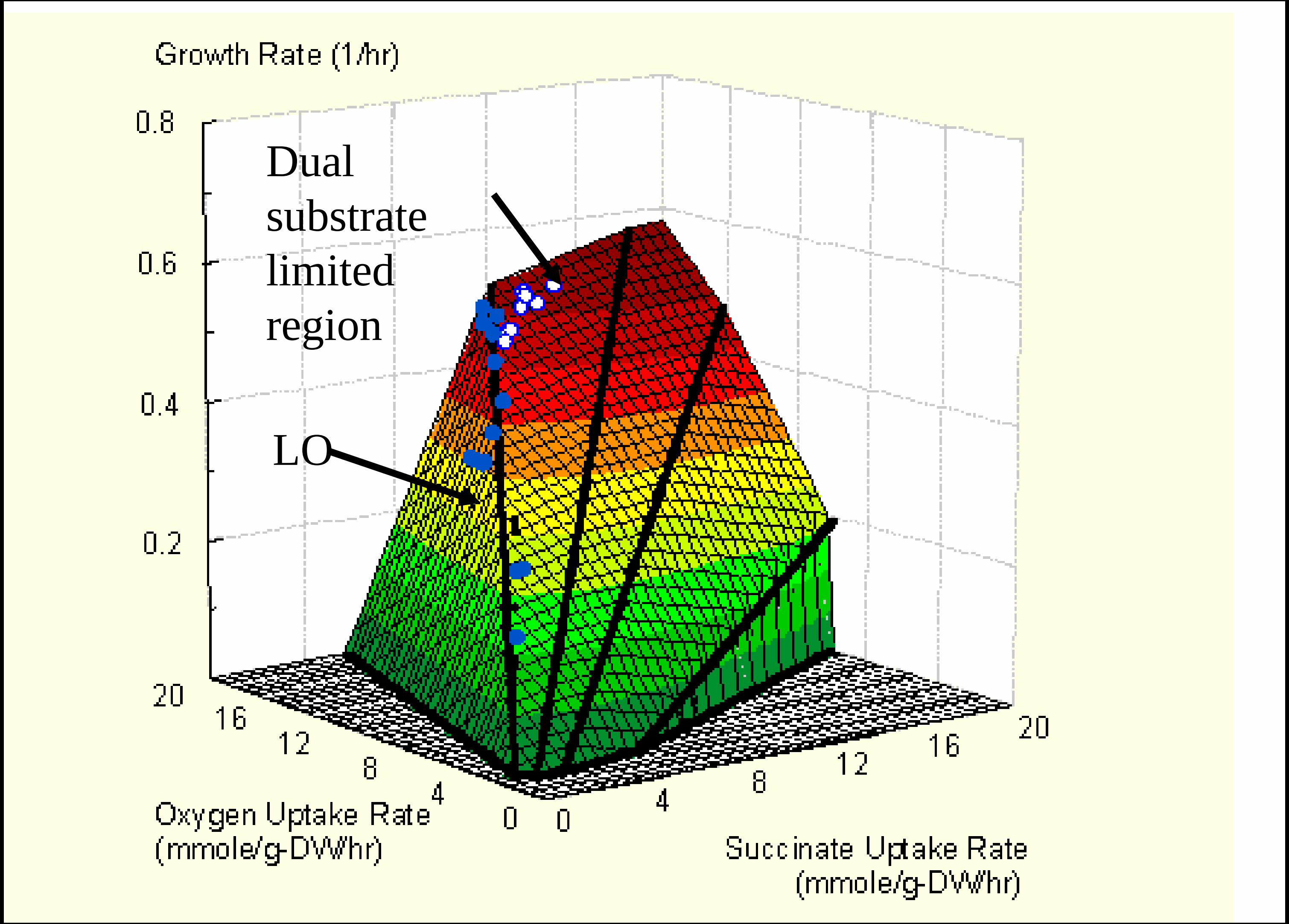
Succinate Phenotype Phase Plane



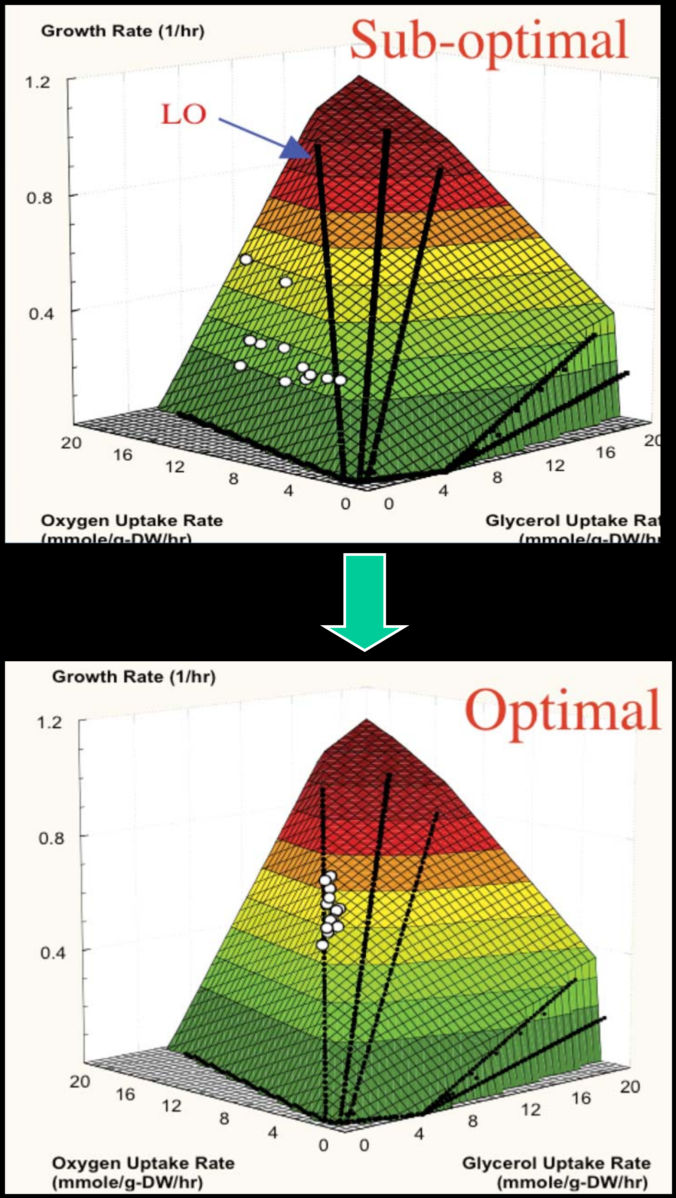
-also works for: malate, glucose, fumarate

-does not work for glycerol

Growth on Succinate

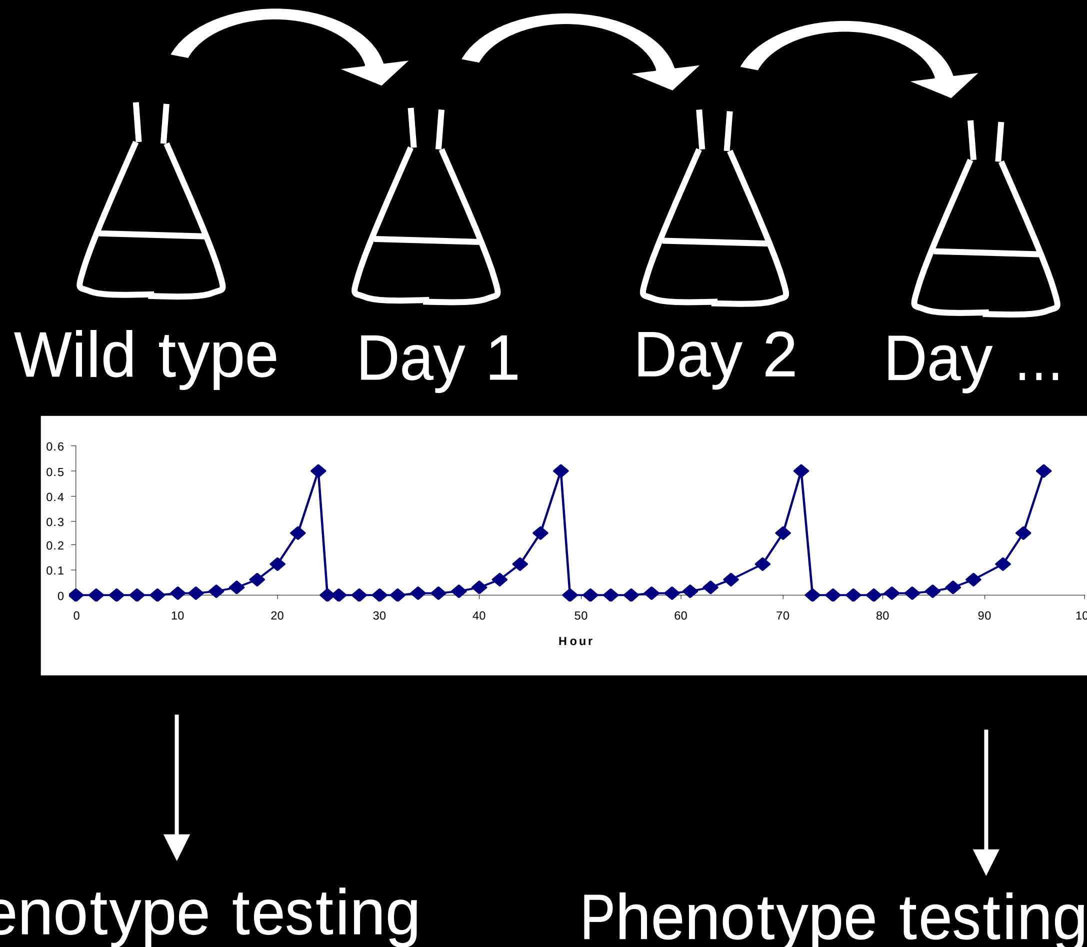


Application: Predicting complex biology; adaptive evolution and picking optimal growth states

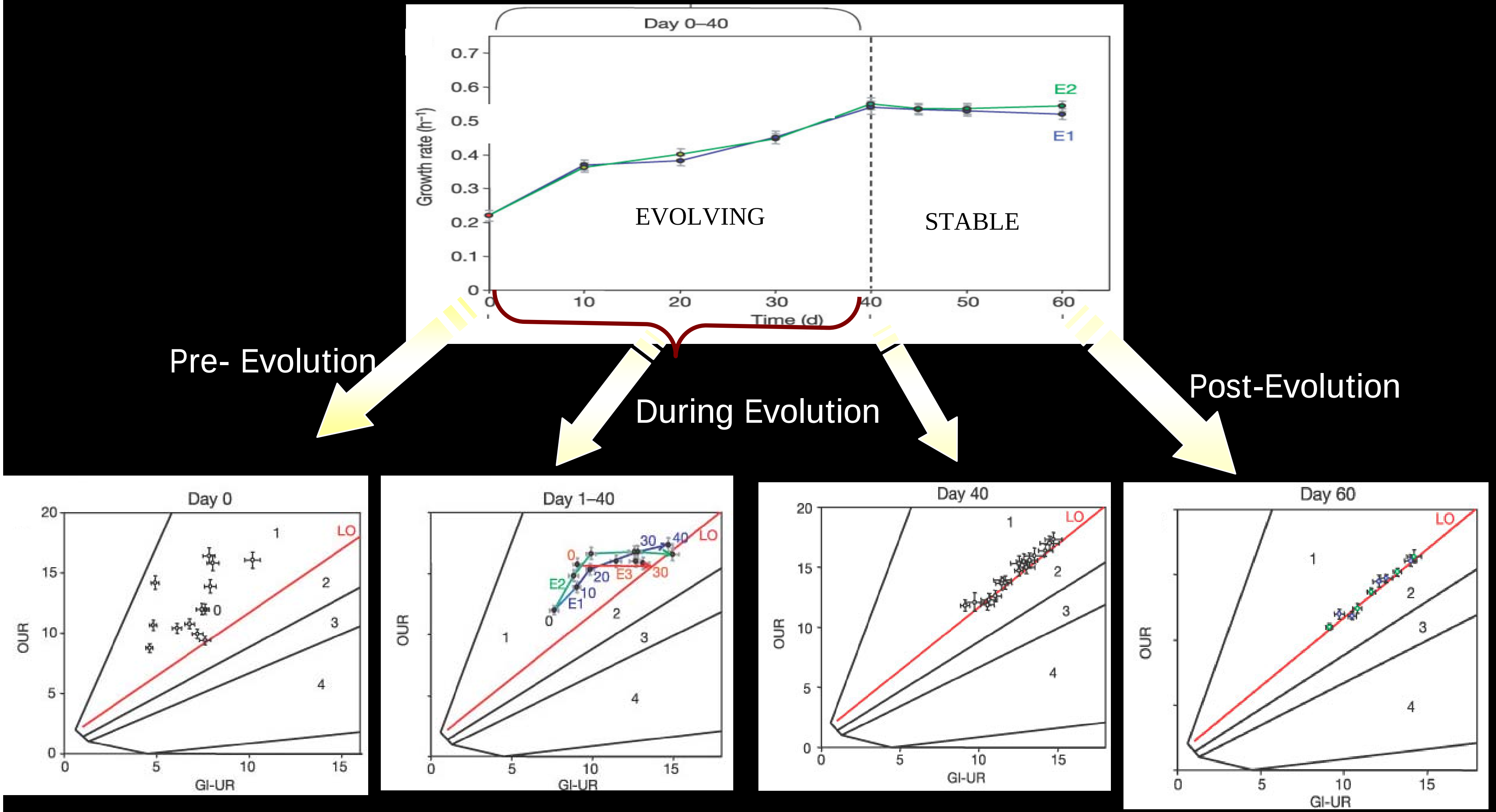


Methods – adaptive evolution

- Cultures grown in 250ml minimal medium supplemented with 2g/L carbon source
- Serial passage during exponential growth
- Stable growth rate achieved at end of evolution
- Cells frozen throughout evolution for phenotype testing



Cellular Evolution: Growth rates on Glycerol



Ibarra et al, 2002, Nature, 420: 186-189