Optimization of Fermentation processes Both at the Process and Cellular Levels

'Simultaneous saccharification and fermentation of starch to lactic acid'

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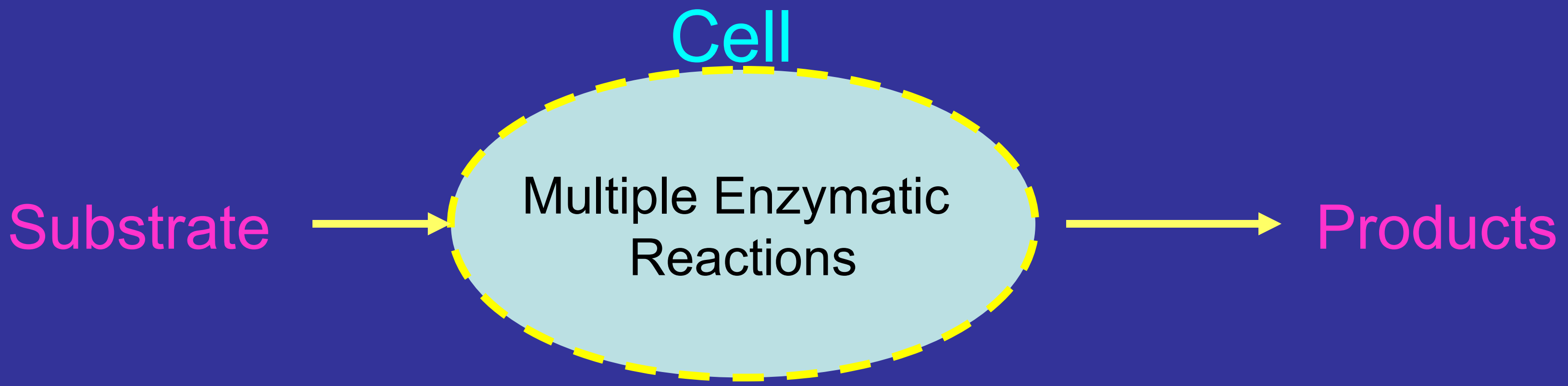
Introduction

- Living cells can be used to produce biochemical products
- Natural screening of the environment to isolate microorganisms
- Isolated microorganisms or cells as pure cultures are grown in bioreactor – fermentation processes
- Fermentation processes are used to produce chemicals ranging from food, polymer, pharmaceuticals, bulk chemicals, bio-energy, waste management etc

Metabolism



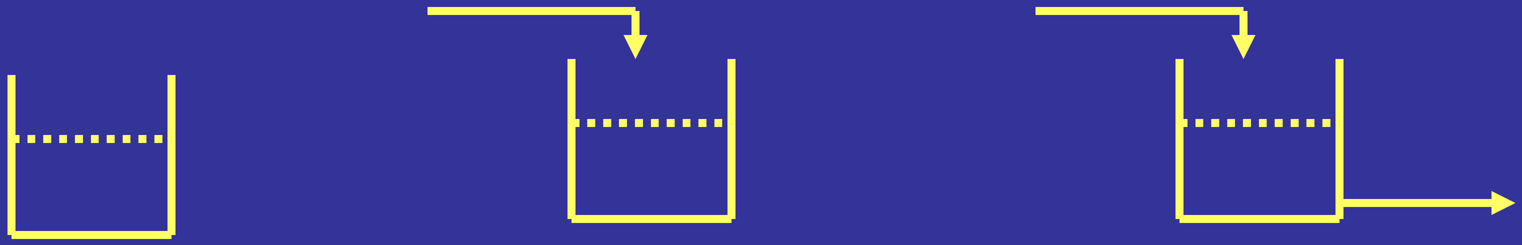
Cell

Substrate → Multiple Enzymatic Reactions → Products

1. Diverse products can be produced by various living cells
2. Diversity depends on the metabolism evolved in a particular organism; Yeast – ethanol, Lactobacillus – Milk to yogurt etc

Fermentation Process

- Cells/Microorganisms require a medium for its growth
- Medium typically contains a carbon, nitrogen and essential nutrients
- Optimal pH and temperature
- Optimization at the process level: Media, environmental conditions
- Operation – batch, fed-batch or continuous



Starch as a carbon Source

- Starch is a polysaccharide of glucose molecules
- Enzymatic hydrolysis to glucose

Inhibits

Alpha - amylase

Gluco-amylase

Starch

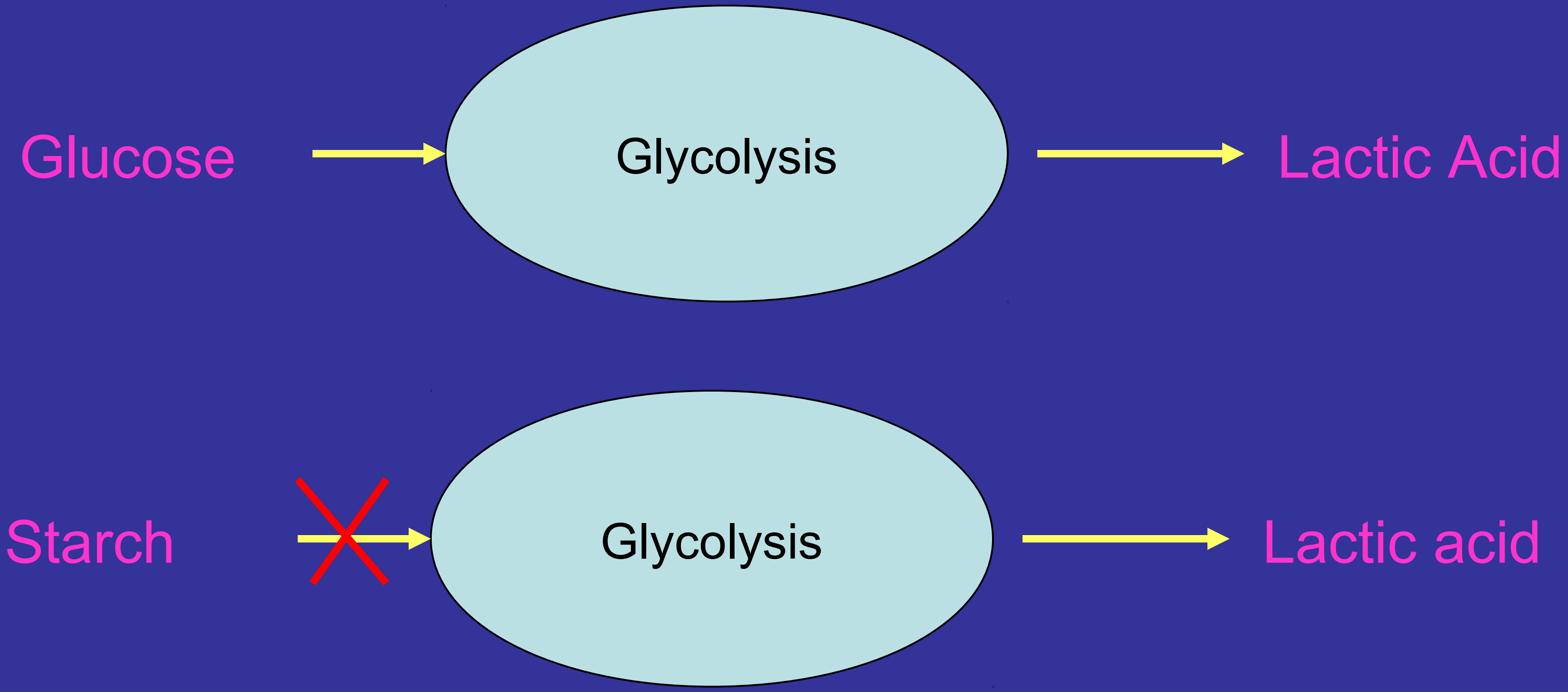
Dextrin

Glucose

Rate of the enzymatic process
is reduced due to glucose inhibition

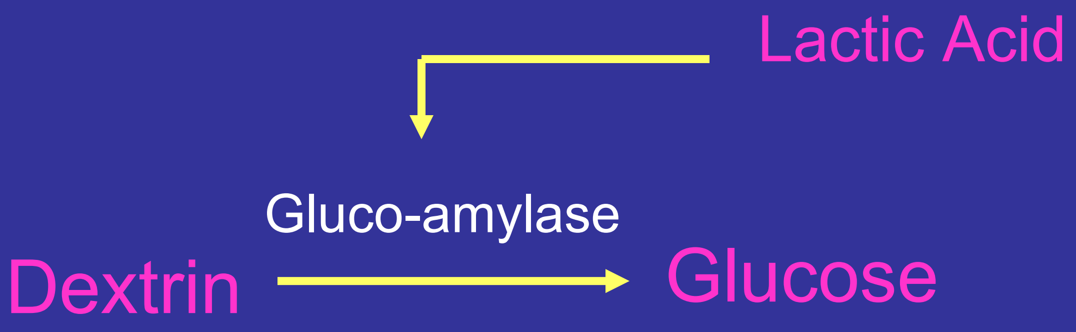
Lactic acid fermentation

- *Lactobacillus* strain converts glucose to lactic acid



Inhibition of Saccharification by fermentative products

- Lactic acid
- Ethanol
- Butanol
- Diacetyl
- Acetoin
- Citric acid


Lactic Acid

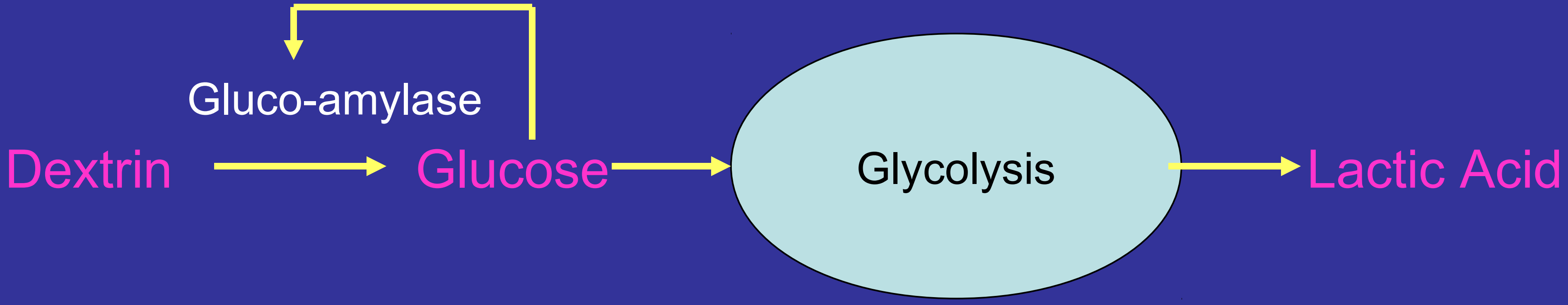
Gluco-amylase

Dextrin

Glucose

The above products offer lesser inhibition than glucose

Simultaneous Saccharification and fermentation



Gluco-amylase

Dextrin → Glucose → Glycolysis → Lactic Acid

Enzymatic reaction and fermentation in the same reactor will not allow the accumulation of glucose to inhibit the saccharification step

Strategy

- Detailed modeling and experimental analysis of the saccharification step
- Detailed modeling and experimental analysis of the fermentation step
- Prediction of optimal condition for SSF using model
- Temperature (45C), pH (5.5) and glucose concentration (< 20 g/L) crucial for the operation of SSF
- Experimental verification to demonstrate increased rates and productivities
- Fed-batch operation for reducing product inhibition

Cellular Optimization

- Cells screened from nature are typically optimized for growth
- Cells are the micro-reactors in the fermentation process
- The main step for economical process is to perform cellular optimization.
- Metabolic and genetic engineering can be used to alter the cellular behaviour.
- Directed mutation versus random mutation.

Metabolic Network analysis

1. Determine the limiting step in the Metabolism
2. Quantification of feasible metabolic space
3. Removal of the limiting step in the network.
4. Detailed Kinetics of the process using metabolism.
5. Elementary mode analysis

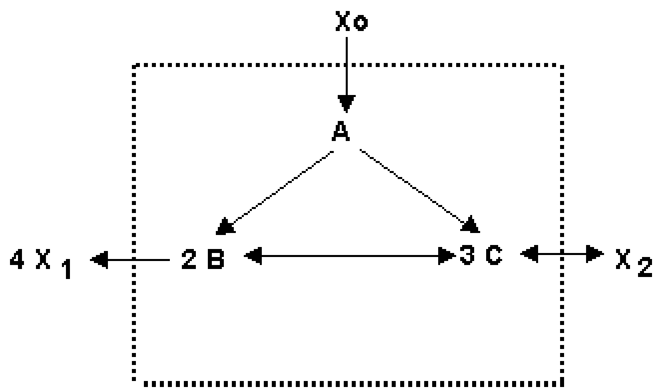
What are the elementary modes ?

An elementary mode is a minimal subset of enzymes in a network that can operate at steady state with all irreversible reactions proceeding in the direction as prescribed by thermodynamics.

Elementary mode analysis links network structure to flux balance (evaluation of reaction rates)

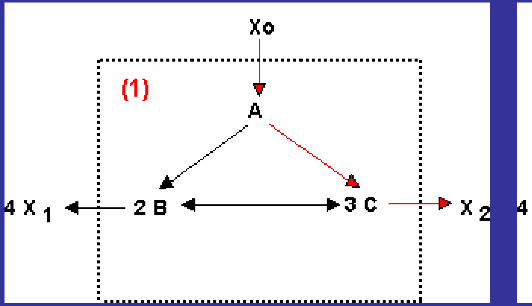
Methodology: Hypothetical Network

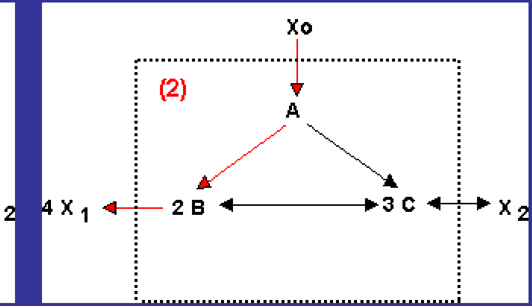
System chosen

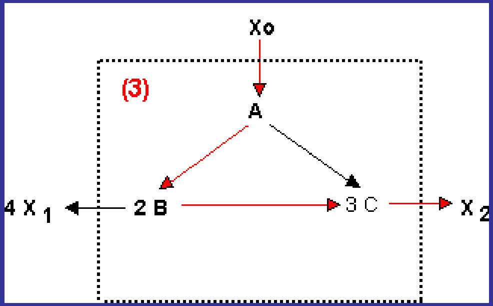


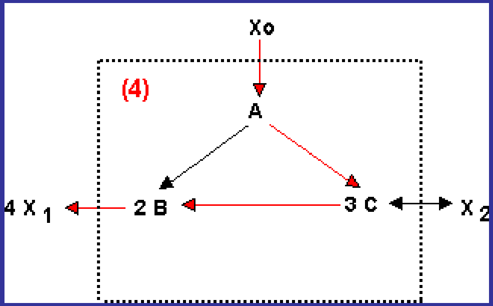
Fluxes of elementary modes	Reaction stoichiometry
v_1	$X_o \rightarrow X_2$
v_2	$X_o \rightarrow 4X_1$
v_3	$X_o \rightarrow X_2$
v_4	$X_o \rightarrow 4X_1$
v_5	$X_2 \rightarrow 4X_1$

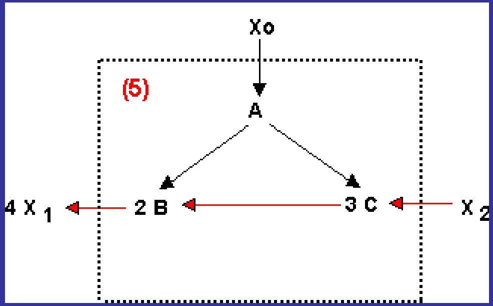
Elementary modes











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Problem formulation

Rates of external metabolites

$$\frac{dX_o}{dt} = v_1 + v_2 + v_3 + v_4$$

$$\frac{dX_1}{dt} = 4v_2 + 4v_4 + 4v_5$$

$$\frac{dX_2}{dt} = v_1 + v_3 - v_5$$

In matrix form

$$\begin{bmatrix} 1 & 1 & 1 & 1 & 0 \\ 0 & 4 & 0 & 4 & 4 \\ 1 & 0 & 1 & 0 & -1 \end{bmatrix} \begin{bmatrix} v_1 \\ v_2 \\ v_3 \\ v_4 \\ v_5 \end{bmatrix} = \begin{bmatrix} dX_o/dt \\ dX_1/dt \\ dX_2/dt \end{bmatrix}$$

Linear programming formulation

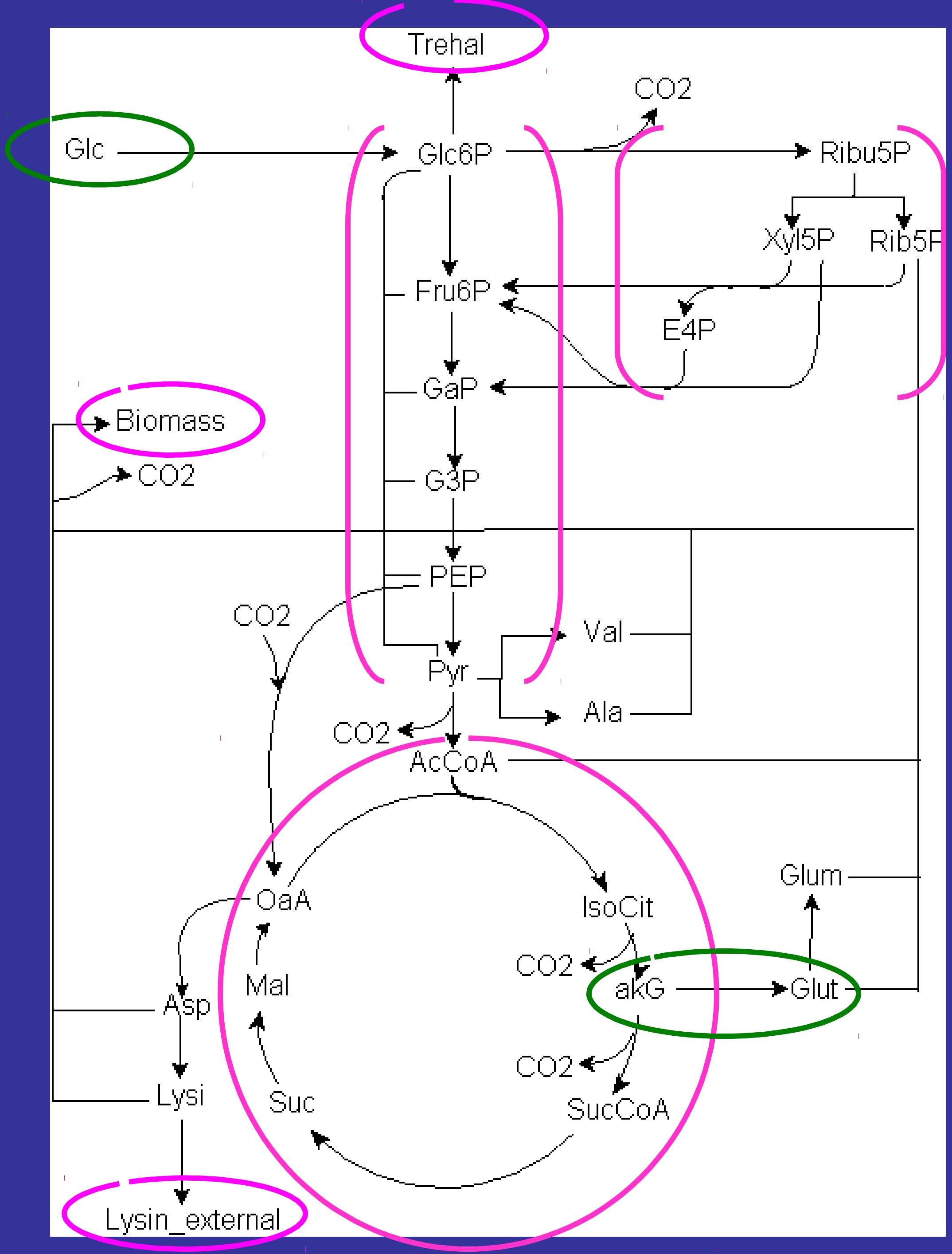
$$\text{Objective function} = \text{maximize } \left(\frac{dX_2}{dt}\right)$$

Subject to

$$\begin{bmatrix} 1 & 1 & 1 & 1 & 0 \\ 0 & 4 & 0 & 4 & 4 \end{bmatrix} \begin{bmatrix} v_1 \\ v_2 \\ v_3 \\ v_4 \\ v_5 \end{bmatrix} = \begin{bmatrix} dX_o/dt \\ dX_1/dt \end{bmatrix}$$

Experimentally Determined (known)

and $0 \le v_i \le \infty$ for all i^{th} elements



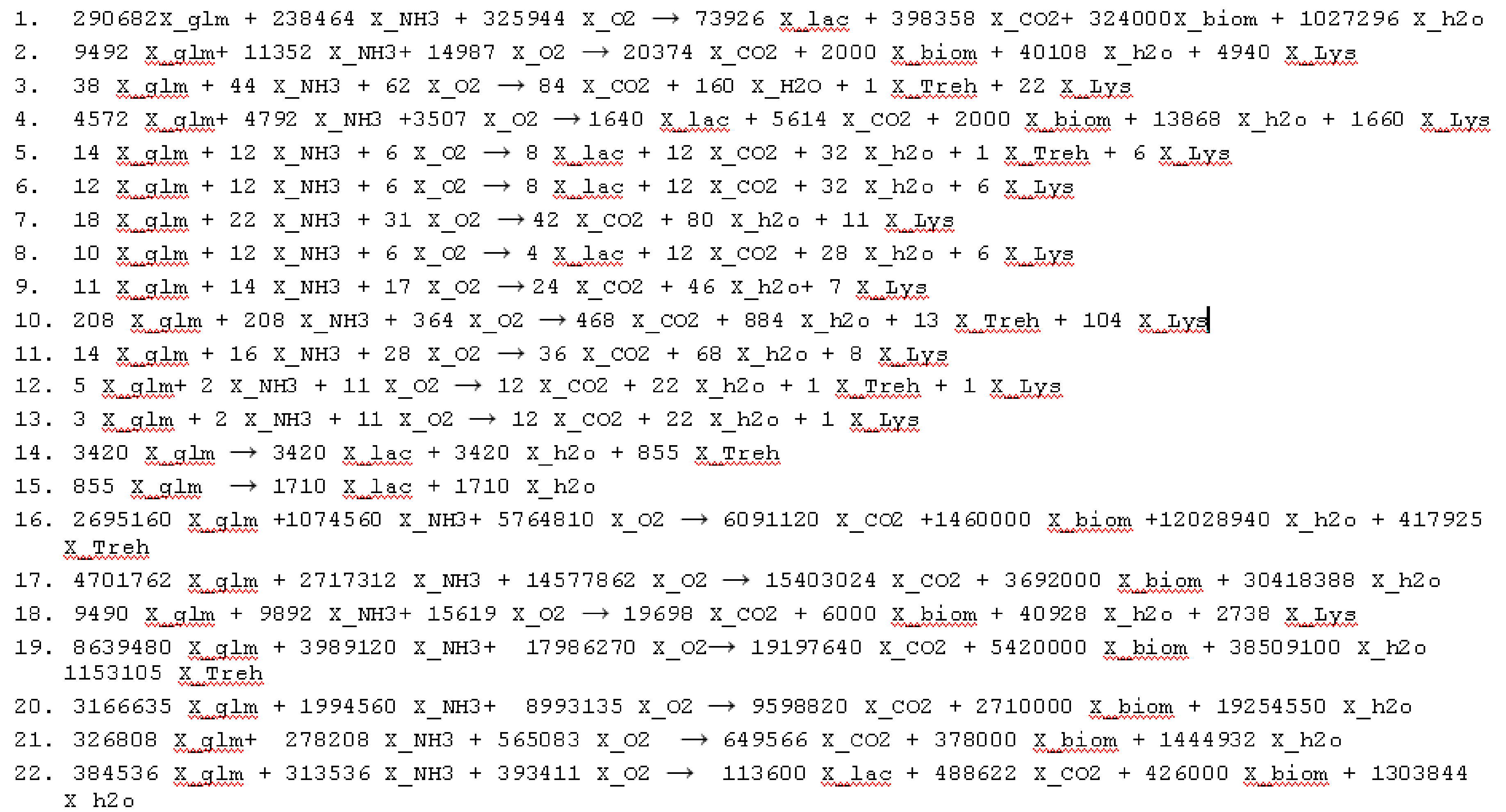
Biochemical Network of *Corynebacterium glutamicum*

Metabolites : 39
Reactions: 40

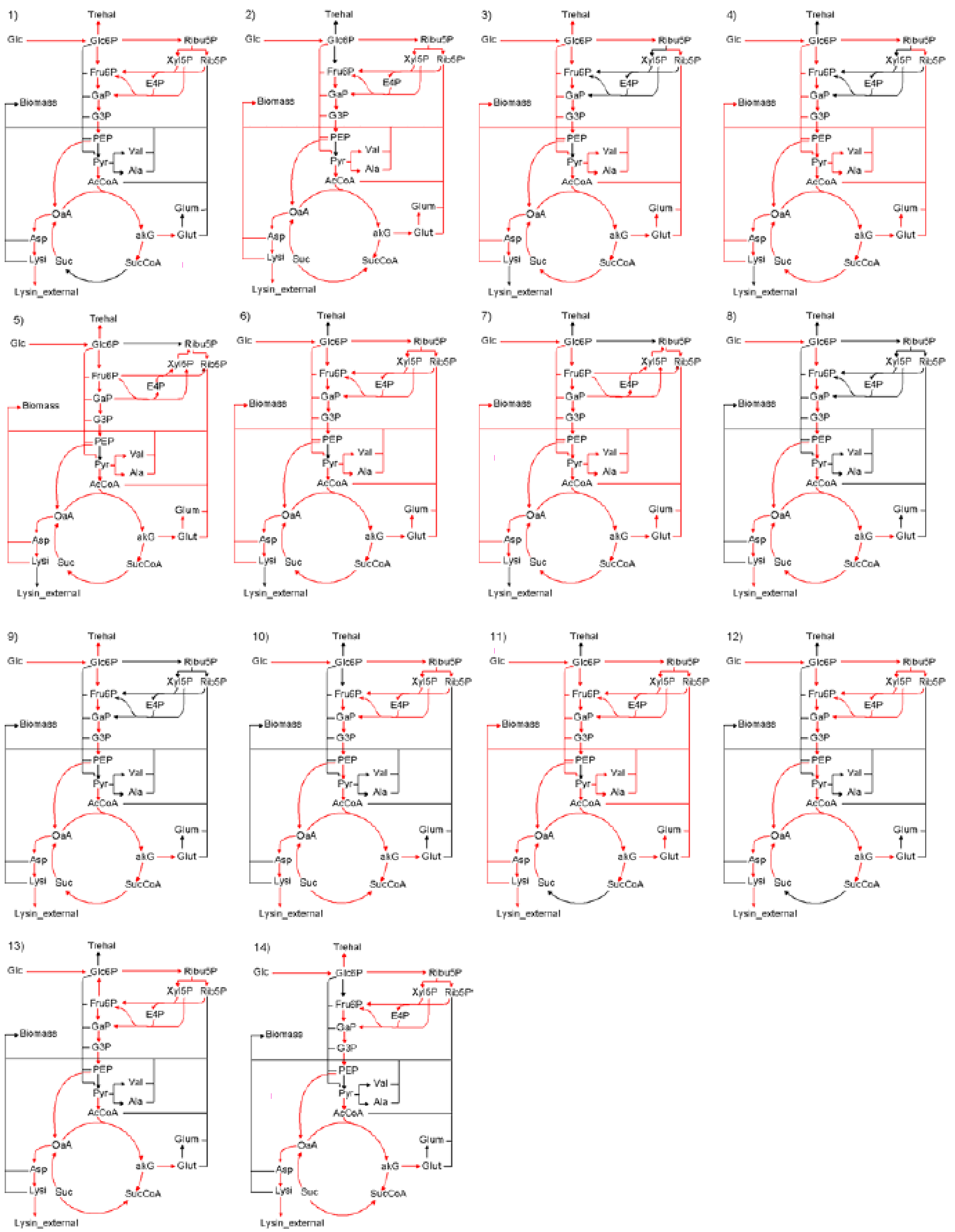
Substrates: Glucose, ammonia and oxygen

Products: Lysine, Biomass, Trehalose and Carbon dioxide

Elementary Modes for the network of *C. glutamicum*



1. 290682X_glm + 238464 X_NH3 + 325944 X_O2 → 73926 X_lac + 398358 X_CO2+ 324000X_biom + 1027296 X_h2o
2. 9492 X_glm+ 11352 X_NH3+ 14987 X_O2 → 20374 X_CO2 + 2000 X_biom + 40108 X_h2o + 4940 X_Lys
3. 38 X_glm + 44 X_NH3 + 62 X_O2 → 84 X_CO2 + 160 X_H2O + 1 X_Treh + 22 X_Lys
4. 4572 X_glm+ 4792 X_NH3 +3507 X_O2 →1640 X_lac + 5614 X_CO2 + 2000 X_biom + 13868 X_h2o + 1660 X_Lys
5. 14 X_glm + 12 X_NH3 + 6 X_O2 → 8 X_lac + 12 X_CO2 + 32 X_h2o + 1 X_Treh + 6 X_Lys
6. 12 X_glm + 12 X_NH3 + 6 X_O2 → 8 X_lac + 12 X_CO2 + 32 X_h2o + 6 X_Lys
7. 18 X_glm + 22 X_NH3 + 31 X_O2 →42 X_CO2 + 80 X_h2o + 11 X_Lys
8. 10 X_glm + 12 X_NH3 + 6 X_O2 → 4 X_lac + 12 X_CO2 + 28 X_h2o + 6 X_Lys
9. 11 X_glm + 14 X_NH3 + 17 X_O2 →24 X_CO2 + 46 X_h2o+ 7 X_Lys
10. 208 X_glm + 208 X_NH3 + 364 X_O2 →468 X_CO2 + 884 X_h2o + 13 X_Treh + 104 X_Lys
11. 14 X_glm + 16 X_NH3 + 28 X_O2 → 36 X_CO2 + 68 X_h2o + 8 X_Lys
12. 5 X_glm+ 2 X_NH3 + 11 X_O2 → 12 X_CO2 + 22 X_h2o + 1 X_Treh + 1 X_Lys
13. 3 X_glm + 2 X_NH3 + 11 X_O2 → 12 X_CO2 + 22 X_h2o + 1 X_Lys
14. 3420 X_glm → 3420 X_lac + 3420 X_h2o + 855 X_Treh
15. 855 X_glm → 1710 X_lac + 1710 X_h2o
16. 2695160 X_glm +1074560 X_NH3+ 5764810 X_O2 → 6091120 X_CO2 +1460000 X_biom +12028940 X_h2o + 417925 X_Treh
17. 4701762 X_glm + 2717312 X_NH3 + 14577862 X_O2 → 15403024 X_CO2 + 3692000 X_biom + 30418388 X_h2o
18. 9490 X_glm + 9892 X_NH3+ 15619 X_O2 → 19698 X_CO2 + 6000 X_biom + 40928 X_h2o + 2738 X_Lys
19. 8639480 X_glm + 3989120 X_NH3+ 17986270 X_O2→ 19197640 X_CO2 + 5420000 X_biom + 38509100 X_h2o 1153105 X_Treh
20. 3166635 X_glm + 1994560 X_NH3+ 8993135 X_O2 → 9598820 X_CO2 + 2710000 X_biom + 19254550 X_h2o
21. 326808 X_glm+ 278208 X_NH3 + 565083 X_O2 → 649566 X_CO2 + 378000 X_biom + 1444932 X_h2o
22. 384536 X_glm + 313536 X_NH3 + 393411 X_O2 → 113600 X_lac + 488622 X_CO2 + 426000 X_biom + 1303844 X h2o



Elementary modes operational in *Corynebacterium glutamicum*

Fourteen elementary modes

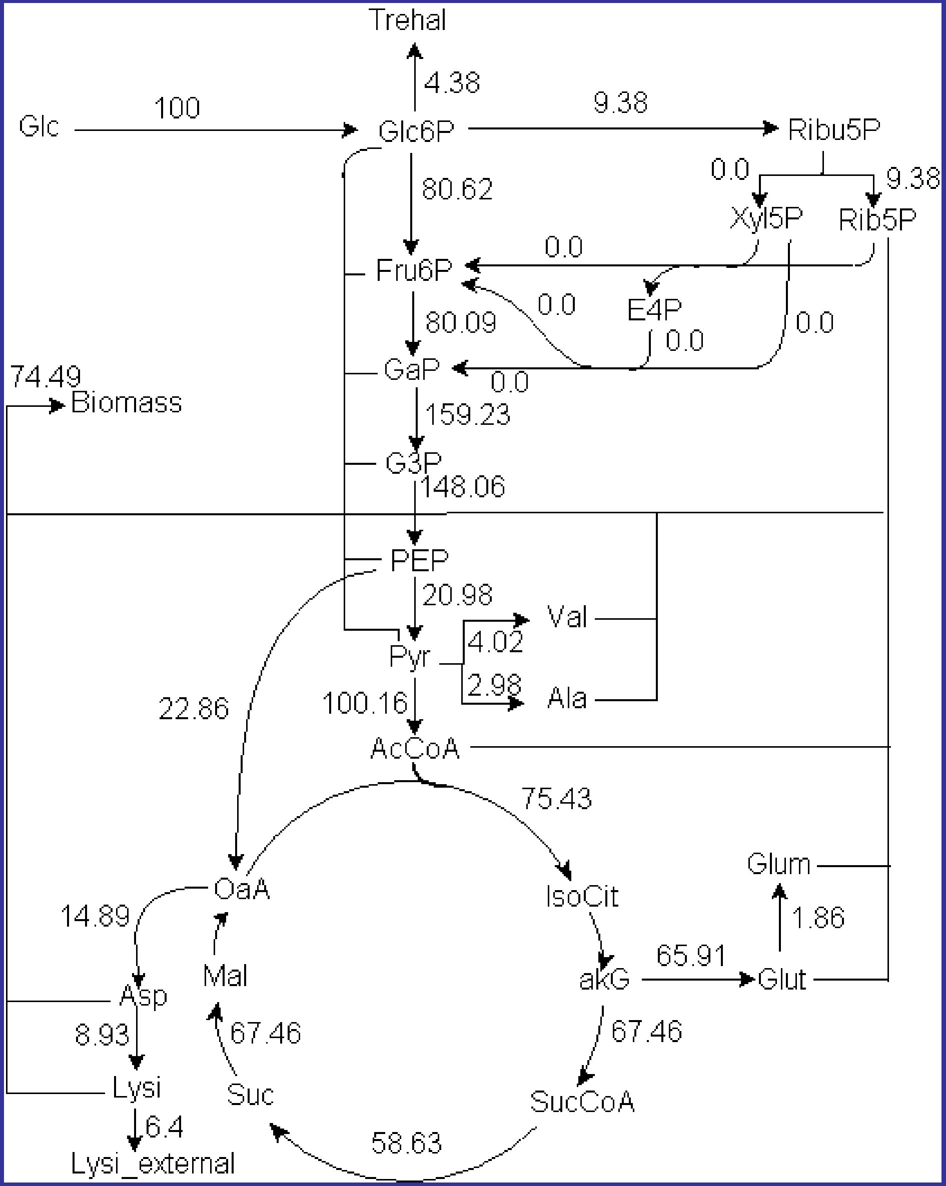
Maximum biomass (124)

Maximum Lysine (63.5)

Kalyan Gayen and K. V Venkatesh
BMC Bioinformatics, 2006

Flux distribution map of *C. glutamicum* for lysine production

Optimal Biomass



Conclusions

- Two level Optimization of fermentation processes – reactor and cellular
- Reactor: Engineering approaches - SSF, fed-batch operation, in situ separation etc
- SSF concept has been used for conversion of cellulose to biofuels
- Cellular: Metabolic Engineering
- Quantification of biological processes is a key step in such an application

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