In silico analysis for the production of higher carbon alcohols using *Saccharomyces cerevisiae*

Balaji Balagurunathan[1], Sudhakar Jonnalagadda[1], Dong-Yup Lee[2,3], Rajagopalan Srinivasan[1,2]

[1]*Institute of Chemical and Engineering Sciences, A*STAR, Singapore,*
[2]*Dept of Chemical and Biomolecular Engineering, National University of Singapore,*
[3]*Bioprocess Technology Institute, A*STAR, Singapore.*

Bioenergy-II Conference
Session : Chemicals from Biomass and Biorefinery Integration

A*STAR ICES
A★STAR

Outline

- Higher carbon alcohols

- Integrated Bioprocess Development

- Genome Scale Metabolic Models and Constraint Based Analysis

- Butanol Case Study

- Results

ICES
A*STAR

Higher Carbon Alcohols

- Alcohols with 4 or more carbon atoms

- Higher energy content and lower hygroscopicity and vapor pressure make them a better fuel additive

- Applications in food and flavor industries

- Applications as solvent and feedstock in industries

Fuel additives

Flavor compounds

**Feedstock / Solvent for Industry**

Production of higher carbon alcohols

- Microbial bioprocesses for the production of higher carbon alcohols
    - Advantages
        - Utilization of renewable resources
        - Environment friendly operation
        - Suitable for Large Scale production
    - Disadvantages
        - Lower yields
        - Toxicity of alcohols to microorganisms
- Bioprocess development
    - Conventional vs. Integrated processes

Bioprocess Development Workflow
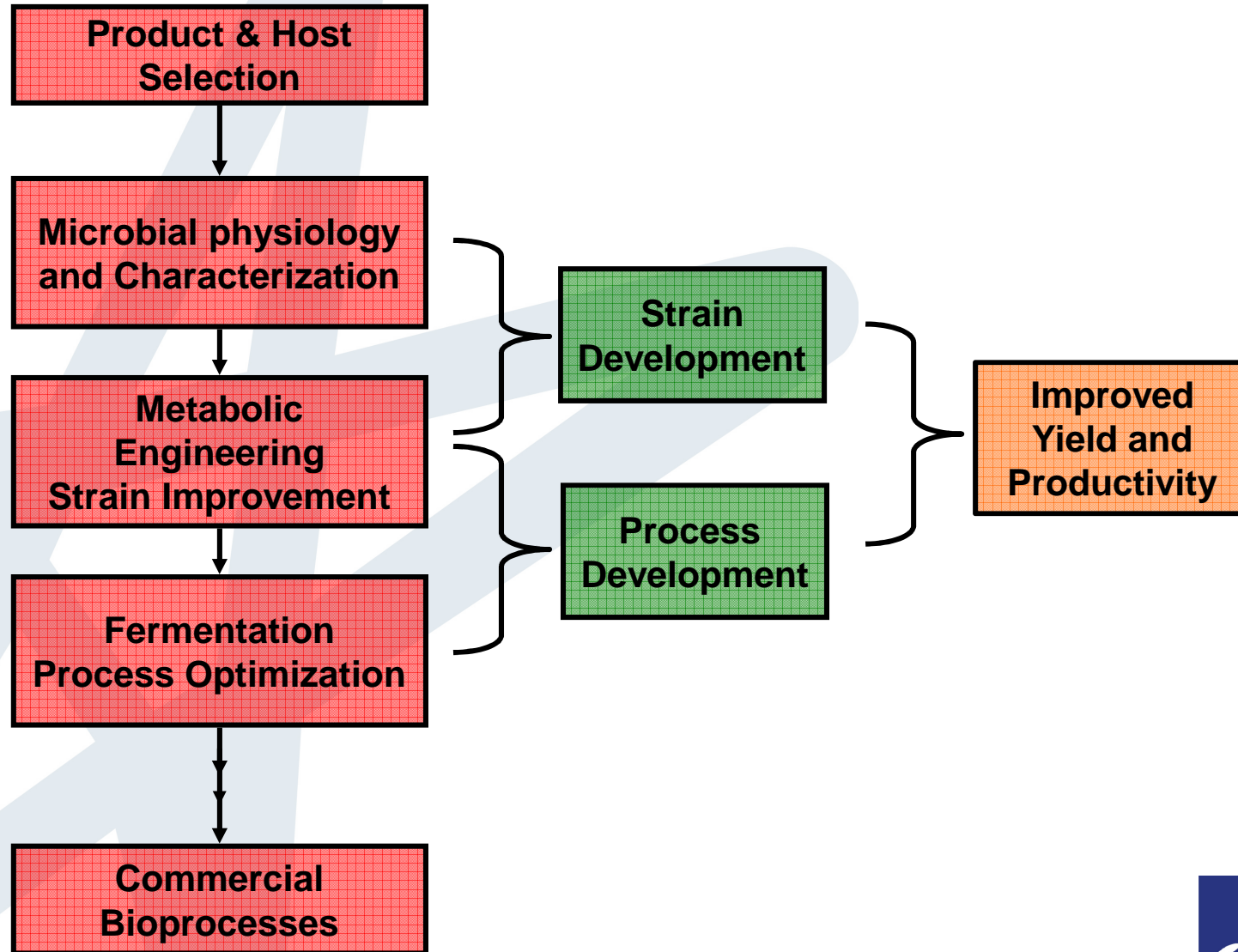
Systems Approach for Integrated Bioprocess Development
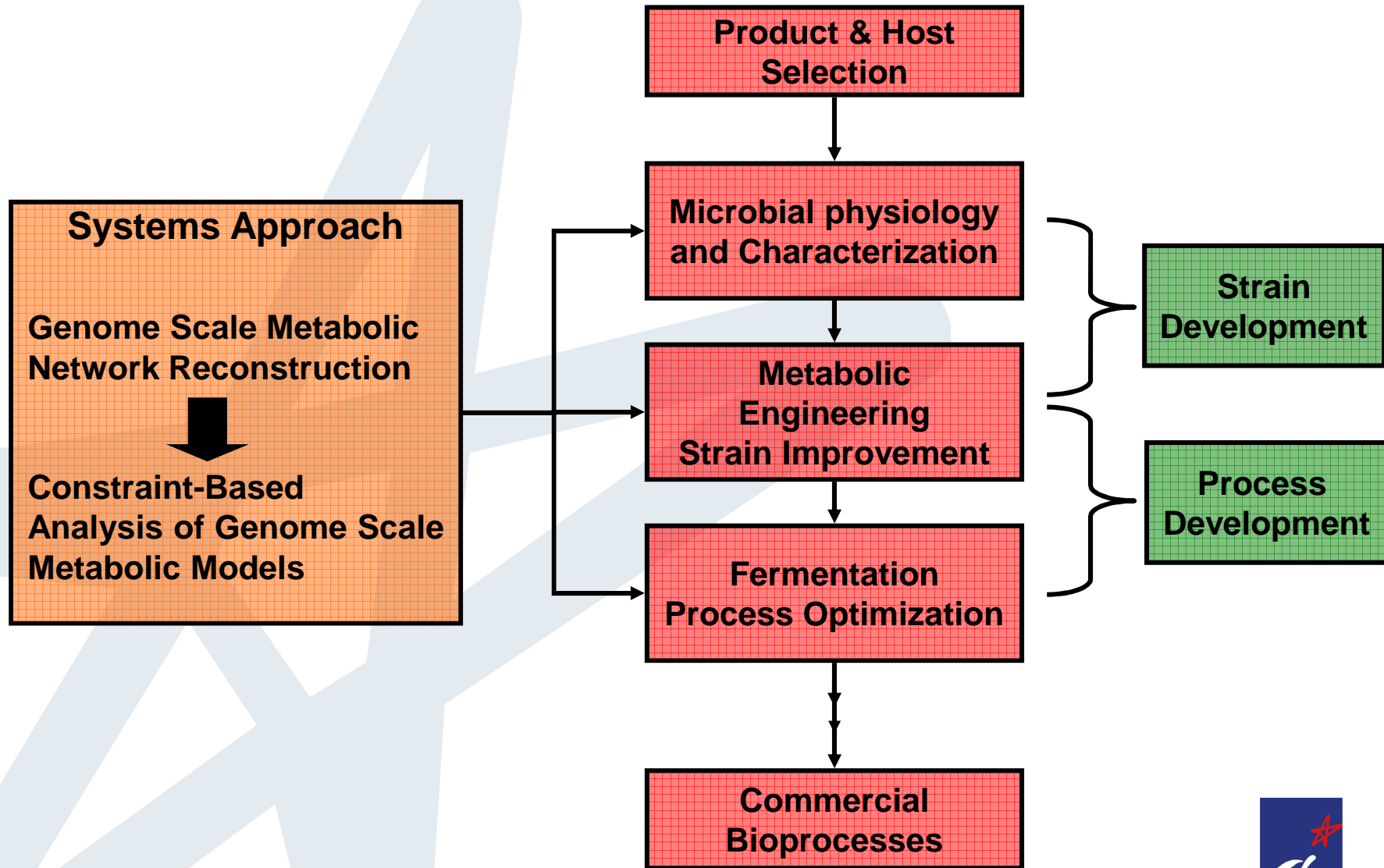
Bioprocess Development Workflow

Genome Scale Metabolic Models

Genome Scale Metabolic Reconstruction (GENRE)

- Genotype →   Phenotype

- Biochemically and genetically structured, highly curated compilation of primary biological information.

- Integration of high-throughput omic and Bibliomic data with small scale detailed experiments

Genome Scale Metabolic Model

- GENRE can be converted to a mathematical model by the application of biological and physico-chemical constraints

- Application of computational methods to assess phenotypic characteristics.
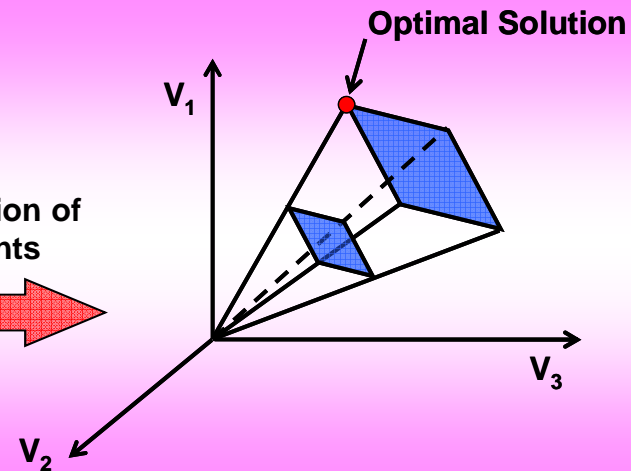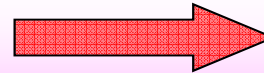
Constraint Based Analysis of Genome Scale Metabolic models

Network Reconstruction

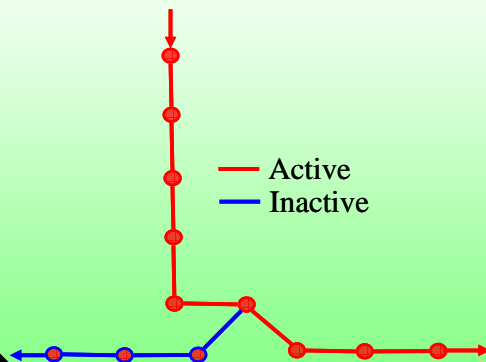Genome sequence, Biochemical Databases, High-throughput data
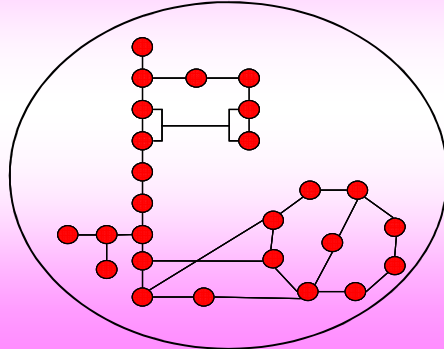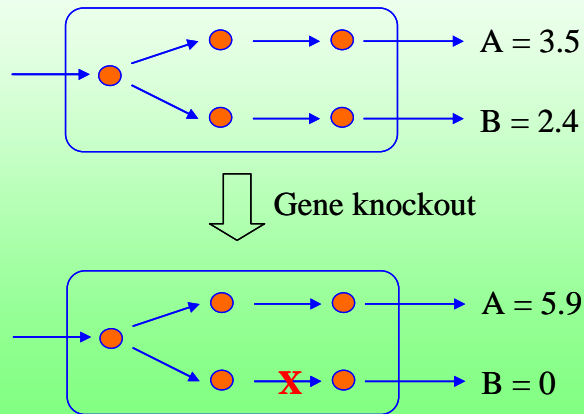
Application of constraints

Optimal Solution

V_1

V_2

V_3

Microbial Physiology and Characterization

- Flux distribution
- Active / Inactive pathways

Active
Inactive

Metabolic Engineering Strain Improvement

- Gene Knockouts
- Gene Insertions

A = 3.5
B = 2.4

Gene knockout

A = 5.9
X
B = 0

Fermentation Process Optimization

- Optimal Operating conditions
- Phenotype Phase Plane analysis

Growth Rate
1.5
1
0.5
0
20
16
12
8
4
0 0
4
8
12
16
20

Glucose Uptake Rate

Oxygen Uptake Rate

ICES
A★STAR
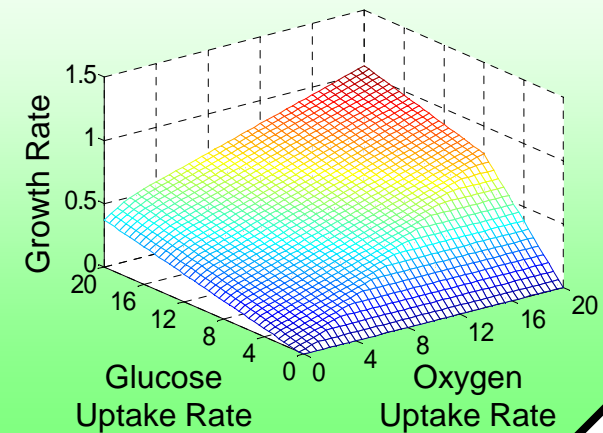
Case Study – Butanol Production

- Selection of microorganism

- Selection of metabolic pathways
    - Fermentative Vs Non-fermentative Pathways

- In silico flux balance analysis (FBA)
    - Tradeoff between growth and product formation
    - Yield of butanol from hexose and pentose sugars
    - In silico gene manipulation studies
        - Gene Deletion
        - Gene Insertion
    - Dynamic FBA

Criteria for the selection of microorganisms

- Micro-organism Selection
    - Yield and Productivity
    - Suitability for Industrial Conditions
    - Ease of genetic manipulation/availability of tools

Organism	Natural sugar utilization pathways					Major products		Tolerance			O_2 needed	pH range
	Glu	Man	Gal	Xyl	Ara	EtOH	Others	Alcohols	Acids	Hydrolysate		
Anaerobic bacteria	+	+	+	+	+	+	+	−	−	−	−	Neutral
E. coli	+	+	+	+	+	−	+	−	−	−	−	Neutral
Z. mobilis	+	−	−	−	−	+	−	+	−	−	−	Neutral
S. cerevisiae	+	+	+	−	−	+	−	++	++	++	−	Acidic
P. stipitis	+	+	+	+	+	+	−	−	−	−	+	Acidic
Filamentous fungi	+	+	+	+	+	+	−	++	++	++	−	Acidic

Pros and cons of various natural microorganisms with regard to industrial ethanol production

Hahn- Hagerdal et al.(2007)Towards industrial pentose-fermenting yeast strains, Appl. Microb. Biotechnol. 74, 937-953

Genome Scale Metabolic Model

Saccharomyces cerevisiae iND750

- 750 genes, 1149 reactions

- All the reactions are both elementally and charge balanced

- 8 Compartments

  [c] : cytosol   [e] : extracellular

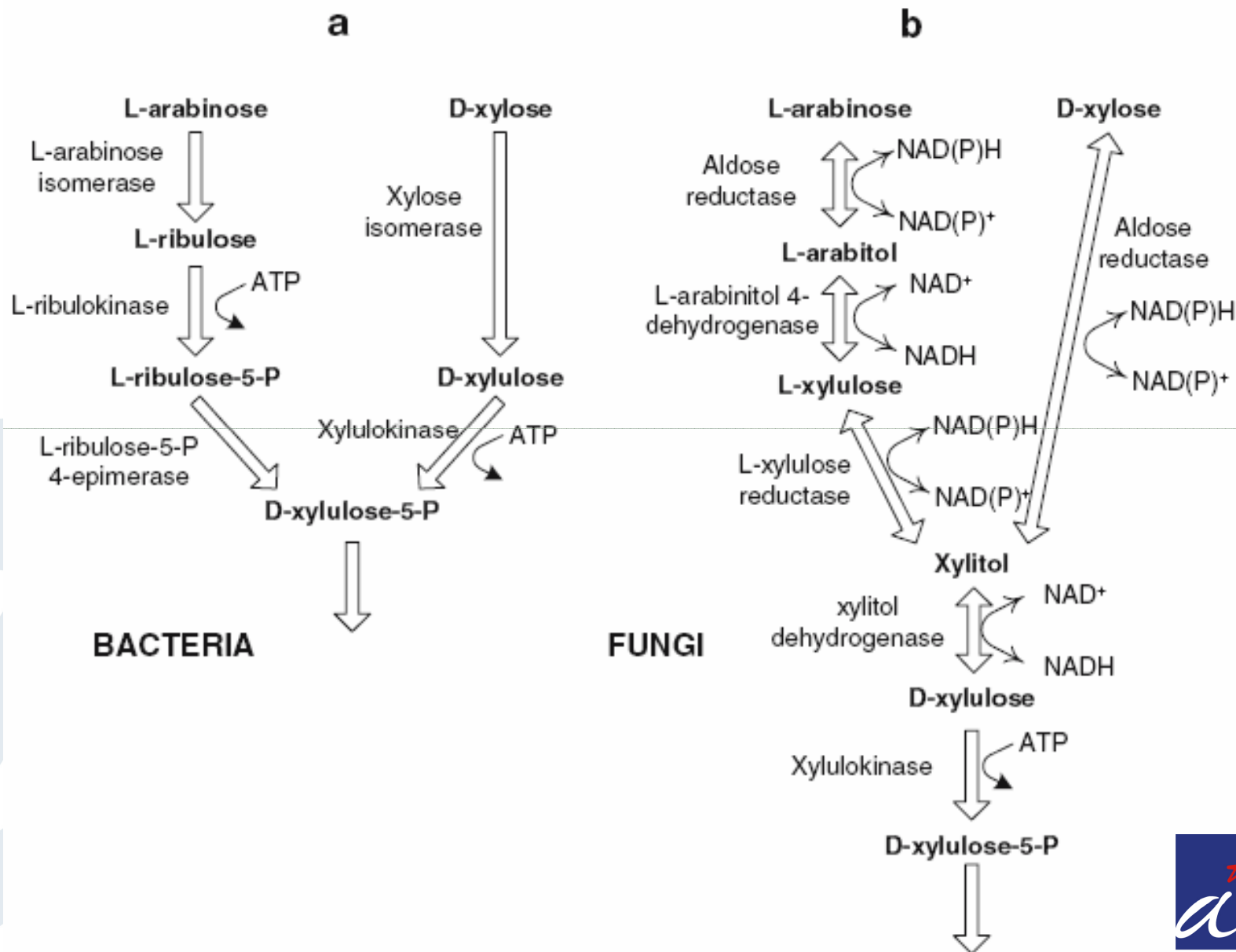 [g] : Golgi apparatus   [m]: mitochondrion

  [n] : nucleus   [r] : endoplasmic reticulum

  [v] : vacoule   [x] : peroxisome
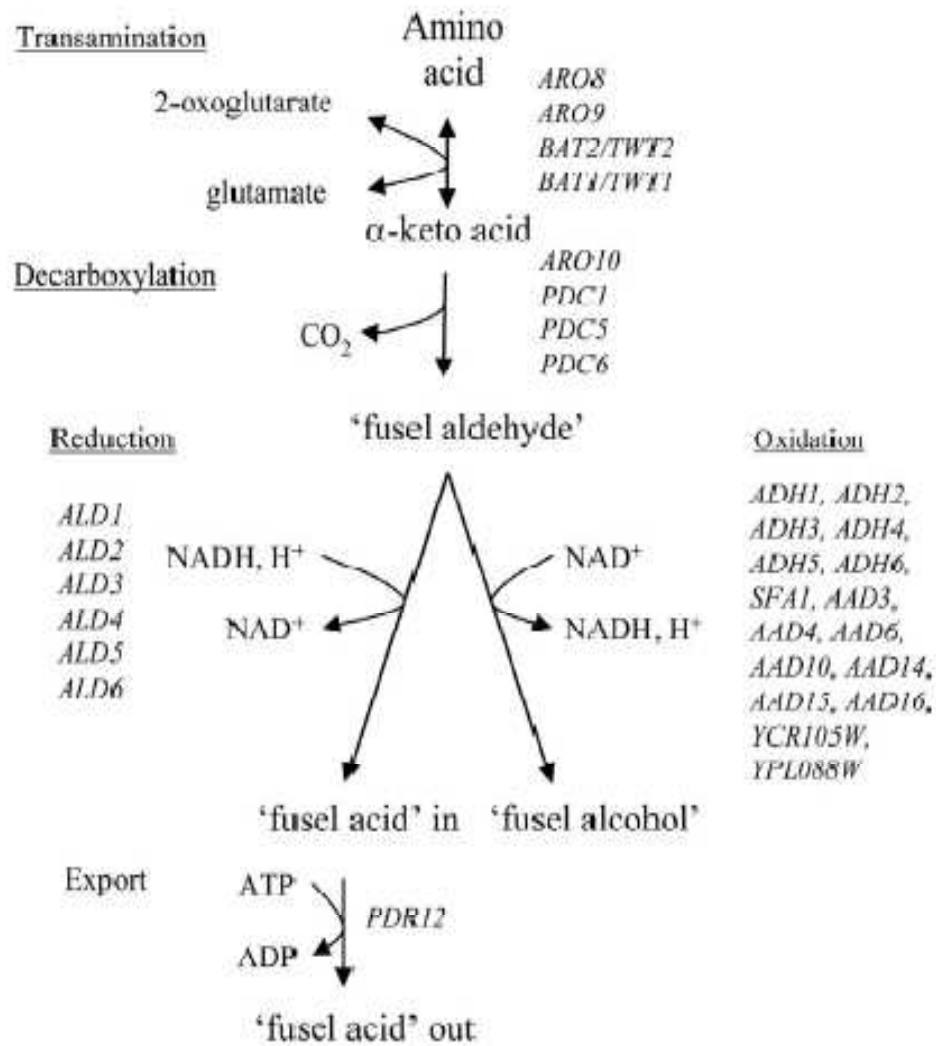
- Current Model contains 750 genes,1266 reactions and 1061 metabolites

ICES

A*STAR

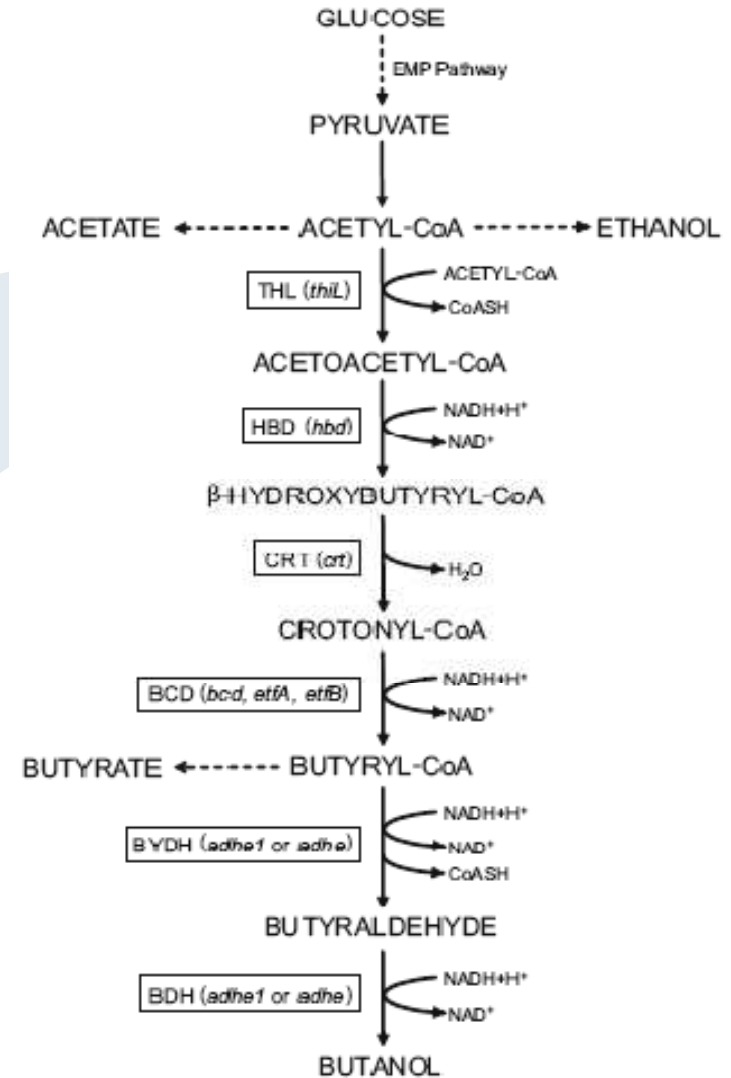# Pathways for Pentose Sugar Utilization

Hagerdal et al., 2007, Appl Microbiol Biotechnol, 74: 937-953

Pathways for butanol production

**Ehrlich Pathway – Non Fermentative**   **Clostridia - Fermentative Pathway**

Butanol Yield- Hexose and Pentose Sugars

Wild Type Model iND750				
Substrate	Growth rate (h^{-1})	Uptake rate mmol/gh	Butanol Yield g/g	Max Yield g/g (Max Theo. Yield 0.411 g/g)
Glucose	0.0858	-5	0.0168	0.185
iND750 + Fungal Xylose Utilization Pathway				
Xylose	0.0712	-5	0	0.181
iND750 + Bacterial Xylose Utilization Pathway				
Xylose	0.0699	-5	0.0137	0.181
iND750 + Bacterial Arabinose Utilization Pathway				
Arabinose	0.0699	-5	0.0137	0.181
iND750 + Fungal Arabinose Utilization Pathway				
Arabinose	0.0802	-5	0	0.181

Growth vs. Product Formation

Biomass vs. Butanol production trade-off
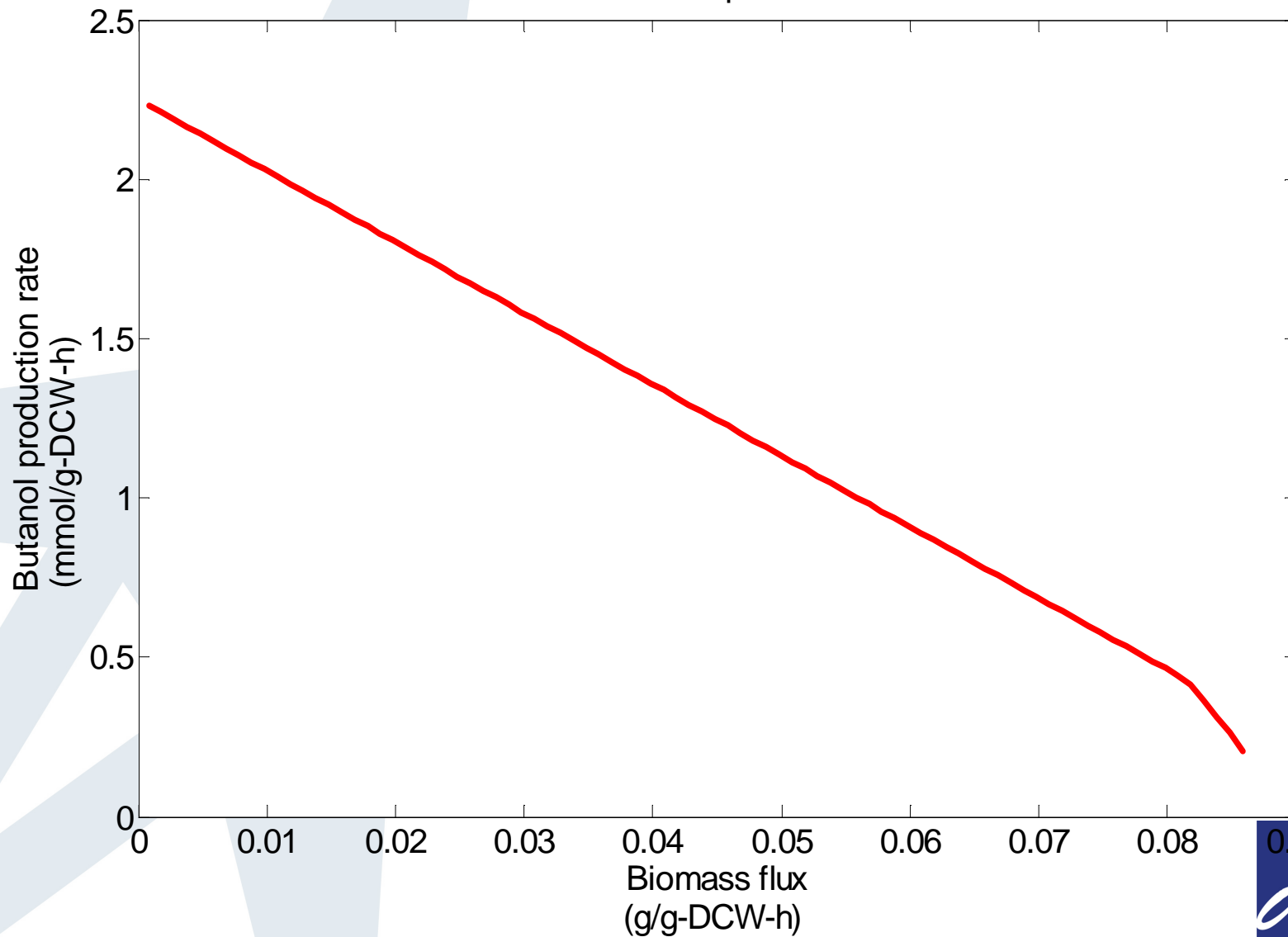
Gene Knockout and Insertion Studies

- Manipulation of cellular metabolism is essential for enhancing the product formation

- Identification of gene targets

- Gene Deletion

  - Double and Triple Gene Knockouts were calculated based on a reduced set of initial genes (which excludes essential genes and genes associated with blocked reactions)

- Gene Insertion

  - Single gene insertion analysis was carried out based on a assembled set of candidate reactions from the KEGG database
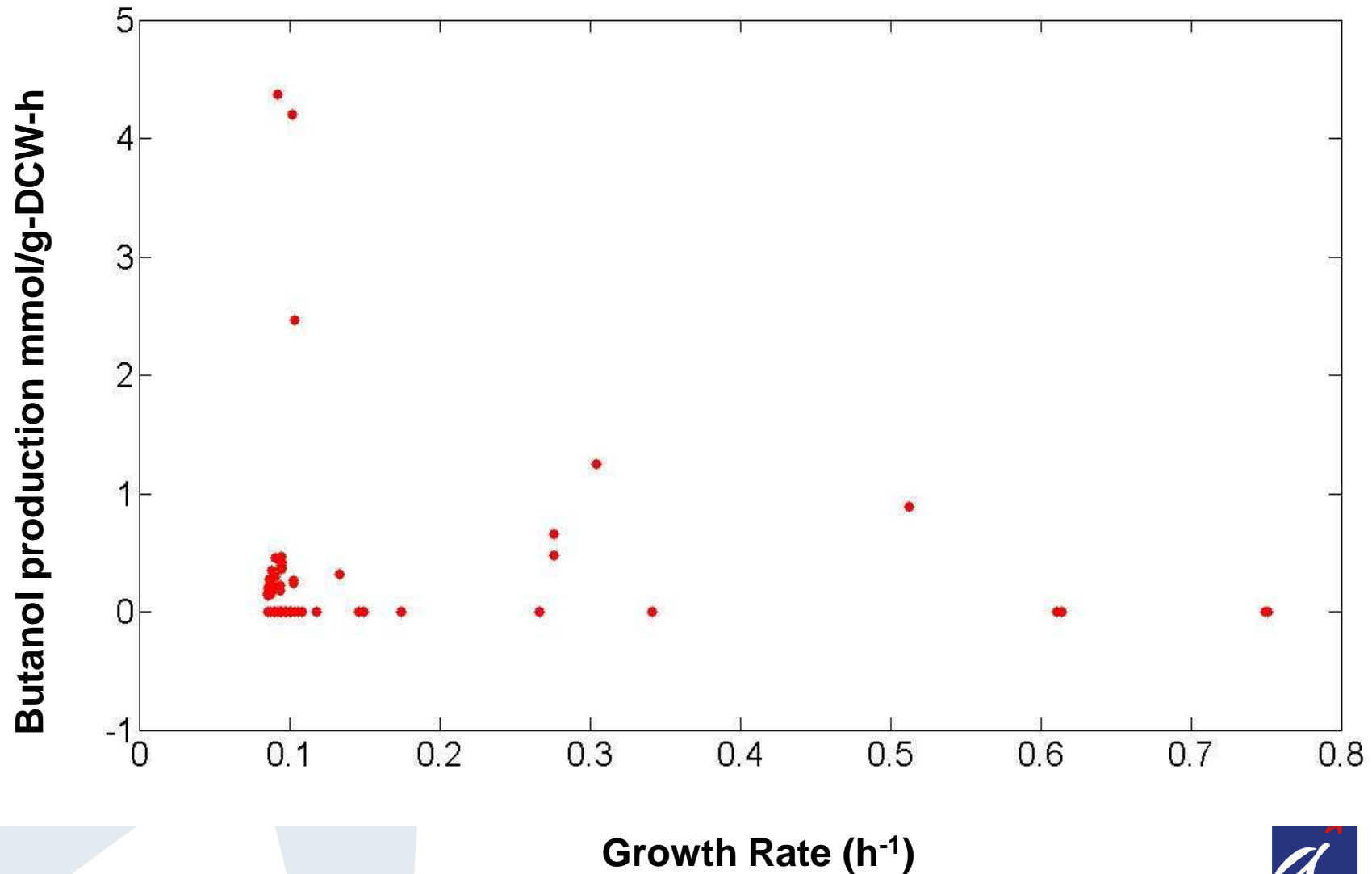
ICES

Gene Deletion studies – Triple Gene Deletion
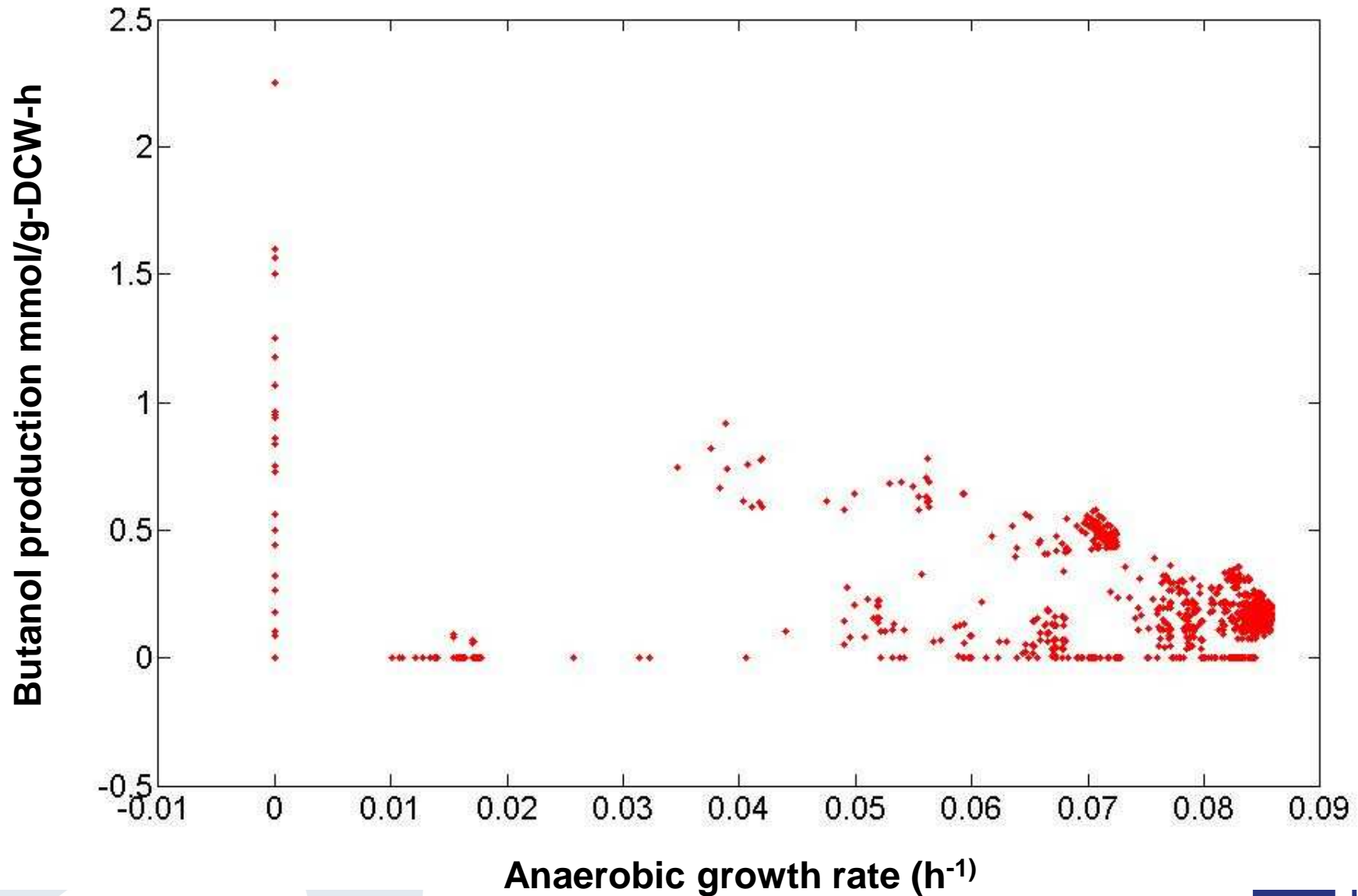
Insertion studies – Single reaction Insertion
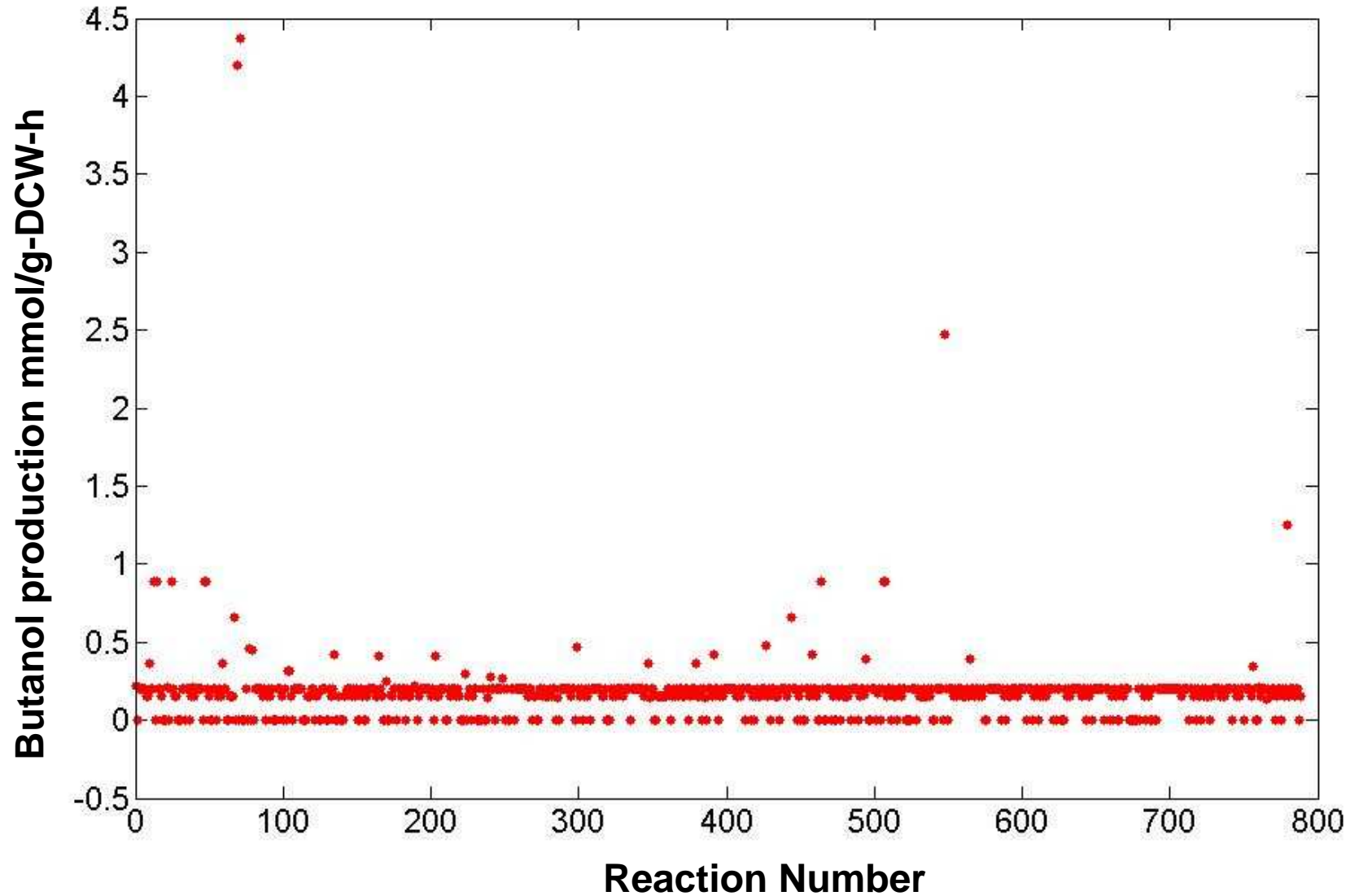
Insertion studies – Single reaction Insertion

**Growth Rate (h⁻¹)**

Dynamic Flux Balance Analysis

- The mathematical model for the process is coupled to the detailed stoichiometric description of cellular metabolism (FBA model)

- The combined model can be used to identify metabolic bottlenecks and gene targets to be manipulated for enhancing the yield

- Additional constraints can be applied to this model to enable integrated strain and process development.
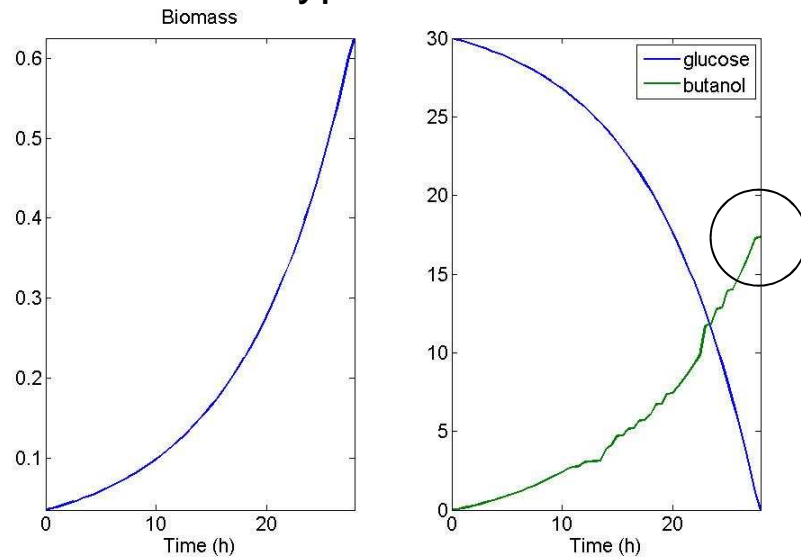
Dynamic Simulation (Dynamic FBA)
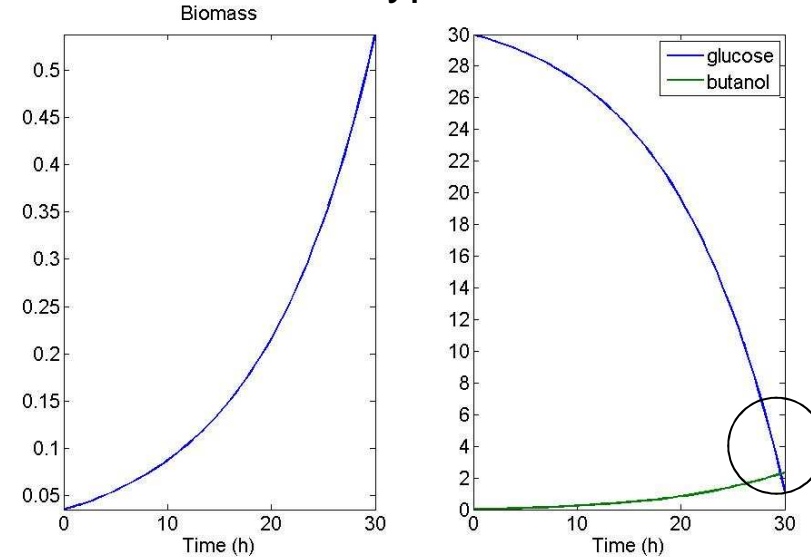

Case I – Wild Type
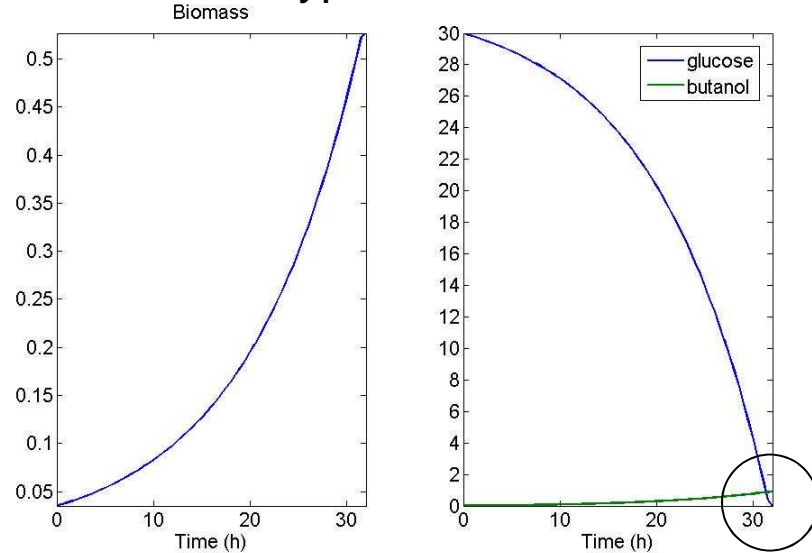
Case 3 - Wild Type + Gene Insertion -2
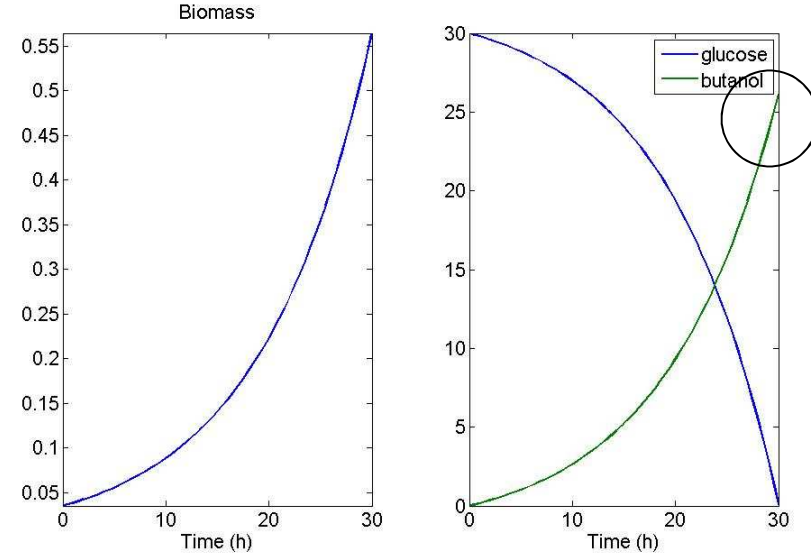
Case 2 – Wild Type + Gene Insertion -1

Case 4 - Wild Type + Gene deletion

Summary

- Systems approach based on genome scale metabolic modeling and analysis has been proposed for Integrated bioprocess development.

- The utility of genome scale metabolic modeling and analysis is demonstrated using the case study for the production of butanol using *Saccharomyces cerevisiae*

  – The metabolic bottlenecks for the production of butanol has been identified by in silico metabolic flux analysis

  – Gene targets to be manipulated for enhancing the yield of butanol has been identified based on gene knockout and gene insertion studies

  – The utility of FBA coupled with dynamic simulation for process development is demonstrated.

ICES

A*STAR

Thank you for the attention…