Modelling & Optimization of Fermentation for Bioethanol Production

Supervisors: Prof. Fraser, Prof. Harrison, Dr. Isafiade

Produced by: Nasibe Nosrati

Chemical engineering department of Cape Town University

Bio-energy

Why biofuels:

- Lack of traditional energy source, (it is estimated that world oil, gas and coal reserves could potentially run out by 2052, 2060, and 2088, respectively at current usage levels)
- Rising fuel costs
- Reducing claim and dependency on fossil supplies which is insecure energy
- Global climate change and global warming

Why bioethanol:

- Sustainable
- Renewable
- Efficient
- Negligible greenhouse gas emissions
- Compatible to the current gas
- Cost-effective energy sources (The financial side of ethanol production by fermentation is notably induced by the charge of raw materials, which accounts for over half of the production price)
- High potential of bioethanol production exists in SA

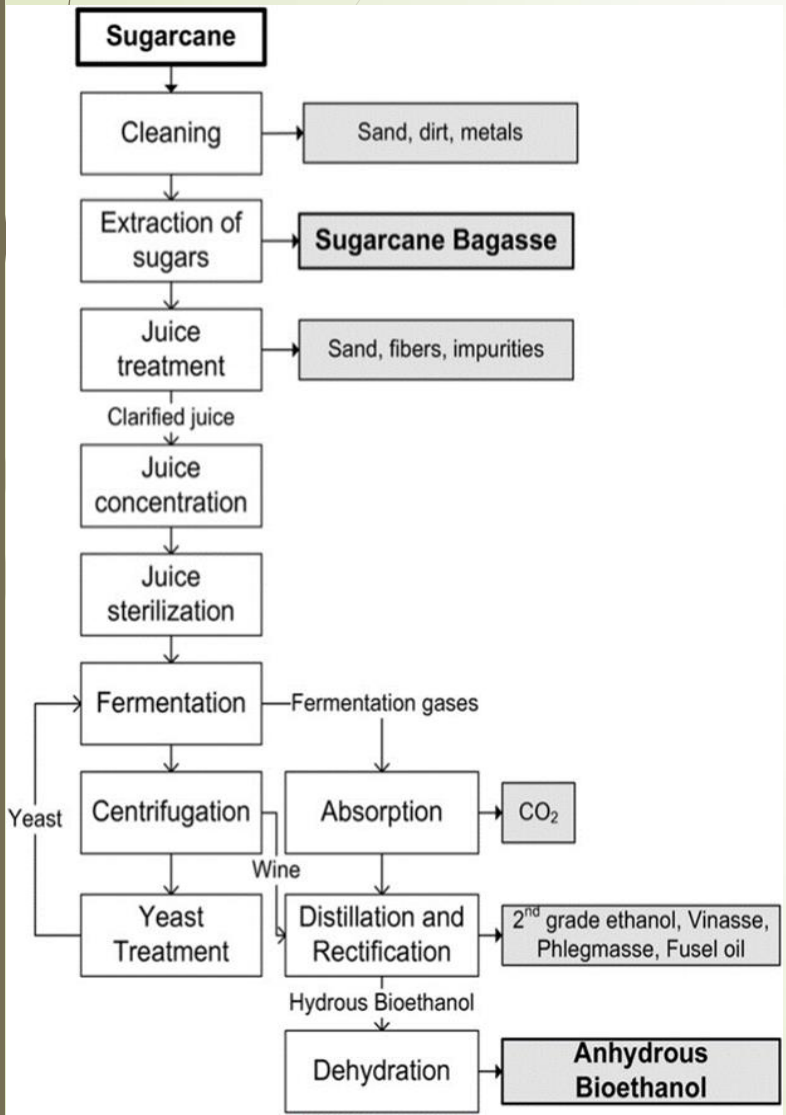
Major producers of bioethanol

Brazil (sugarcane), Canada (Corn (75%)wheat(24%)), USA (corn), Europe (canola) into bio-diesel in Europe, and China (sweet potatoes and cassava)

World Fuel Ethanol Production by Country or Region (Million Gallons)							
Country	**2007**	**2008**	**2009**	**2010**	**2011**	**2012**	**2013**
USA	6521.00	9309.00	10938.00	13298.00	13948.00	13300.00	13300.00
Brazil	5019.20	6472.20	6578.00	6921.54	5573.24	5577.00	6267.00
Europe	570.30	733.60	1040.00	1208.58	1167.64	1179.00	1371.00
China	486.00	501.90	542.00	541.55	554.76	555.00	696.00
Canada	211.30	237.70	291.00	356.63	462.30	449.00	523.00
Rest of World	315.30	389.40	914.00	984.61	698.15	752.00	1272.00
WORLD	**13123.10**	**17643.80**	**20303.00**	**23310.91**	**22404.09**	**21812.00**	**23429.00**

Data Source: F.O. Licht, cited in Renewable Fuels Association, Ethanol Industry Outlook 2008-2013 reports. Available at www.ethanolrfa.org/pages/annual-industry-outlook

Process of bioethanol production



Process of bioethanol production:

- Pre-treatment (breakdown the cell walls)
- Hydrolysis (convert cellulose to sugars)
- Fermentation (convert simple sugars to bioethanol)
- Distillation and dehydration (purify ethanol)

Methods of process:

- Separate hydrolysis and fermentation (SHF): in which the hydrolysis of the cellulose occurs separately to the fermentation
- Simultaneous saccharification and fermentation (SSF): in which the hydrolysis and fermentation of the cellulose occurs together
- Separate hydrolysis and co-fermentation (SHCF): in which both the hemicelluloses sugars (pentose) and cellulose sugars (hexose) are fermented simultaneously after a separate hydrolysis
- Simultaneous saccharification and co-fermentation (SSCF): in which both the hemicelluloses and the cellulose are hydrolysed and fermented simultaneously
- Consolidated bioprocessing (CBP): in which enzyme production, hydrolysis and fermentation of all sugars occurs in one step

Objective

The purpose of this research is to develop models that can be used to optimize the fermentation process for production of bioethanol in greater quantity, higher purity, at optimal levels of economics and environmental impacts, at lab and industrial scales.

Scope of study

- Using metabolic pathway model instead of black box model for optimization of fermentation for bioethanol production from sugarcane.
- Validating the draft model by experimental work.
- Optimizing the developed model using GAMS/Matlab so as to obtain optimal levels of ethanol cost and productivity.

Context

The following parameters will be considered in the optimization of the fermentation process:

- Microorganism among mesophilic and thermophilic
  - Selecting the best range for pH based on selected microorganism, and selecting suitable acid which has less impact on process to maintain constant pH.
  - Selecting the best range of temperature based on type of microorganism
- Kinds of Bioreactor(Batch, Fed-batch and Continuous and then test the effect of immobilization on them),
- Substrate concentration
- Retention time

Determination of output (e.g. ethanol, byproducts, cell dry weight, inhibitors, residues sugar) will be calculated.

Research approach

- Finding metabolic pathway of hexose sugars using saccharomyces cervisiae.
- Modelling metabolic pathway of hexose sugars using saccharomyces cervisiae.
- Achieving operating conditions such as optimal temperature, retention time and sugar concentration.
- Checking metabolic pathways for other microorganisms to see how the parameters in the obtained pathway for saccharomyces cervisiae will change.
- Developing the black box models for each of the different types of reactor from the results of the metabolic pathway model.
- Determining the output concentration, reducing sugars and inhibitors.
- Validating the results of modelling through experimental works and then making changes in the modelling part.
- Combining model with a model developed by other researchers of the group.
- Optimizing the model based on economic and environmental considerations.

Fermentation Process

Aerobic vs Anaerobic:

- Fermentations can either be Aerobic or Anaerobic.
- Anaerobic conditions are more conducive to the production of ethanol.
- Aerobic conditions are more favourable to the production of biomass.

Anaerobic: Ethanol yield: 0.37 biomass yield: 0.05

Aerobic: Ethanol yield: 0.01 biomass yield: 0.35

Reference: Pitkänen et al (2002) (*S. cerevisiae*)

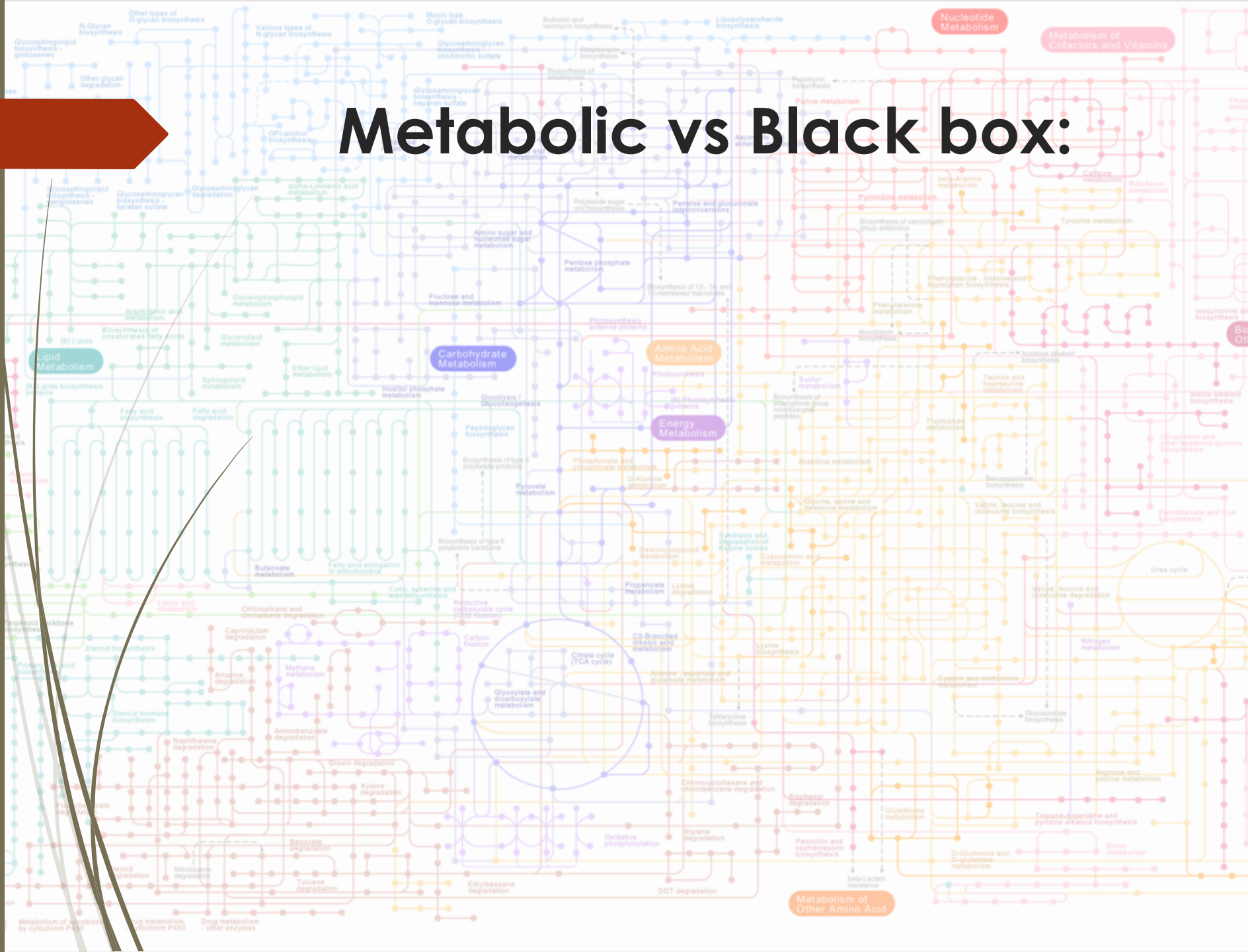
Advantages and disadvantages of various microorganisms

Microorganisms		Advantages	Disadvantages
Mesophiles (20-45 °C)	Saccharomyces cerevisiae	- High ethanol concentrations - Tolerate a wide variety of inhibitors - Tolerate a raised osmotic pressure	-Ferment only hexose sugars -Microbial contamination
	Zymomonas mobilis	- Higher bioethanol yield than yeast - higher tolerances for changes in pH and temperature than yeast	- Ferment only glucose, fructose and sucrose - Less hardy than yeast
	Escherichia coli	- Its growth requires a narrow pH range (6.0–8.0) - Ability to convert both C6 and C5 sugars	- Less hardy than yeast - Ethanol is not the major product
	Kluyveromyces marxianus	- Ability to convert both C6 and C5 sugars - High temperature tolerance to 47° C (suitable for SSF methods) - Energy savings	
Thermophiles (45-122° C)	Thermophilic anaerobic bacteria	- Ability to convert both C6 and C5 sugars - Ability to endure fluctuations in pH, and temperature - Less microbial contamination than yeast - Decrease energy input	- The production of high ethanol yields (>90% theoretical) and high ethanol concentration (>40 g/l) is impossible to be obtained

Description of reactors:

- Batch reactor are used to handle low volume and high value product.
- Fed-batch reactor might be the only option for toxic or low solubility substrates.
- Sequencing batch reactor is proposed in waste water treatment processes.
- Immobilized cell reactor has considerably higher productivity than other reactors due to high cell density and immobilization.

Metabolic vs Black box:



Black box:

Black Box models do not require biochemical information. It is possible to measure the incoming and exit flows of substrates and products to calculate rates of production and consumption. In fact, black box models cannot be extrapolated, they are difficult to interpret, and do not provide analyses of the reactor behavior.

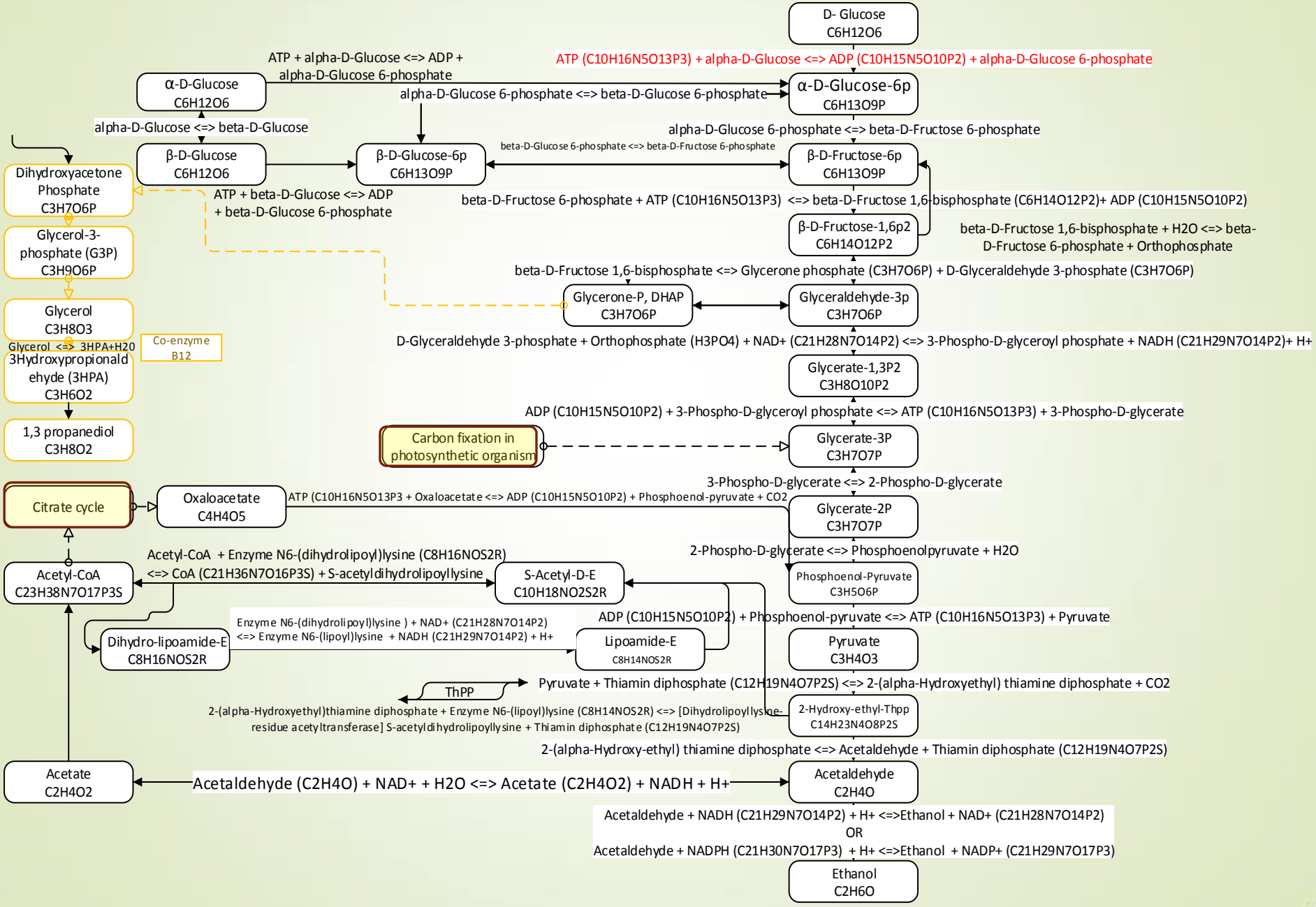
Metabolic Pathway:

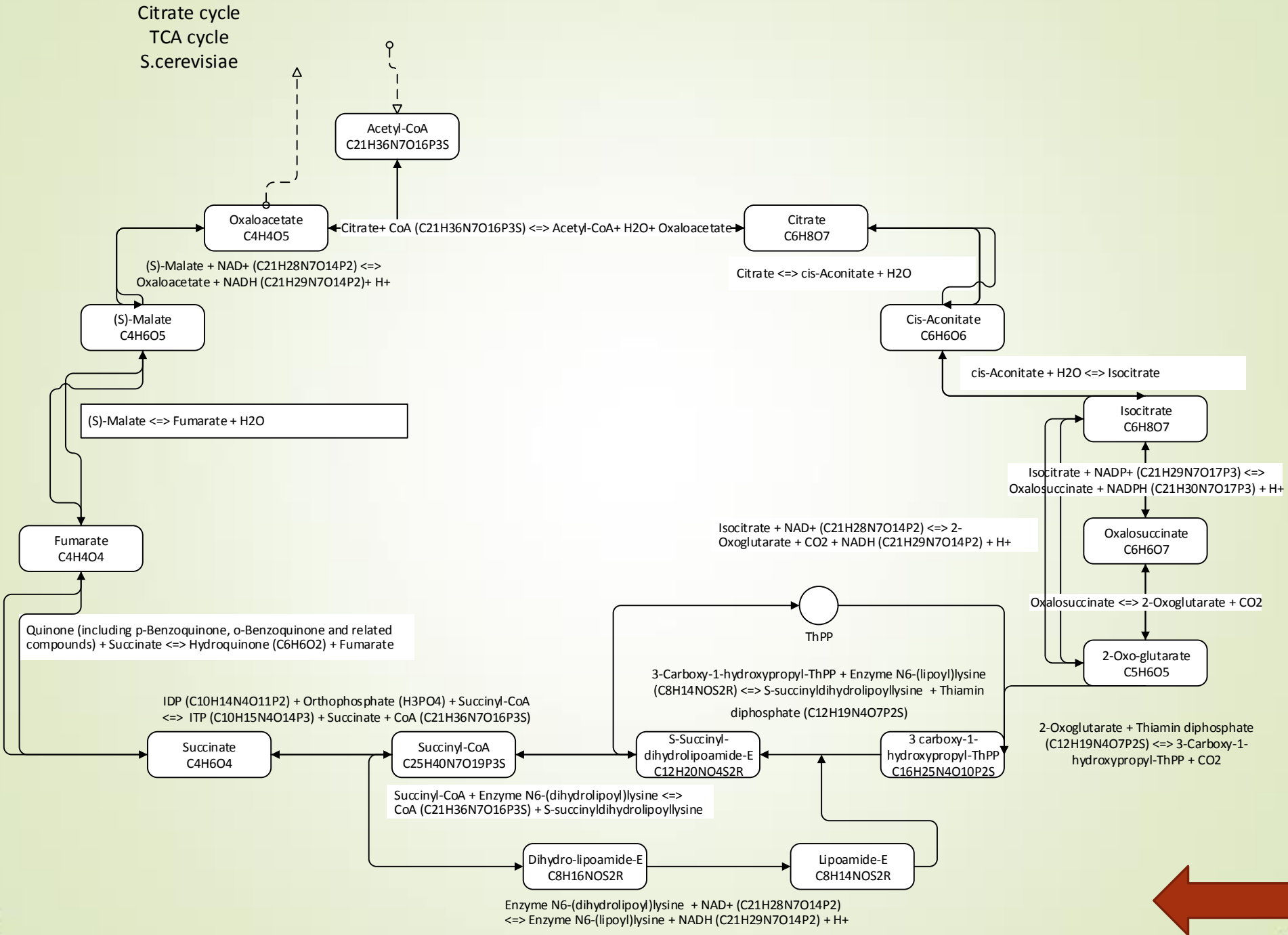
Metabolic pathway models provide a more rigorous method for calculating the consumption of substrate and production of products. Compared to a black box model, which only uses a conversion factor, the metabolic pathway model provides an exceptional amount of detail.

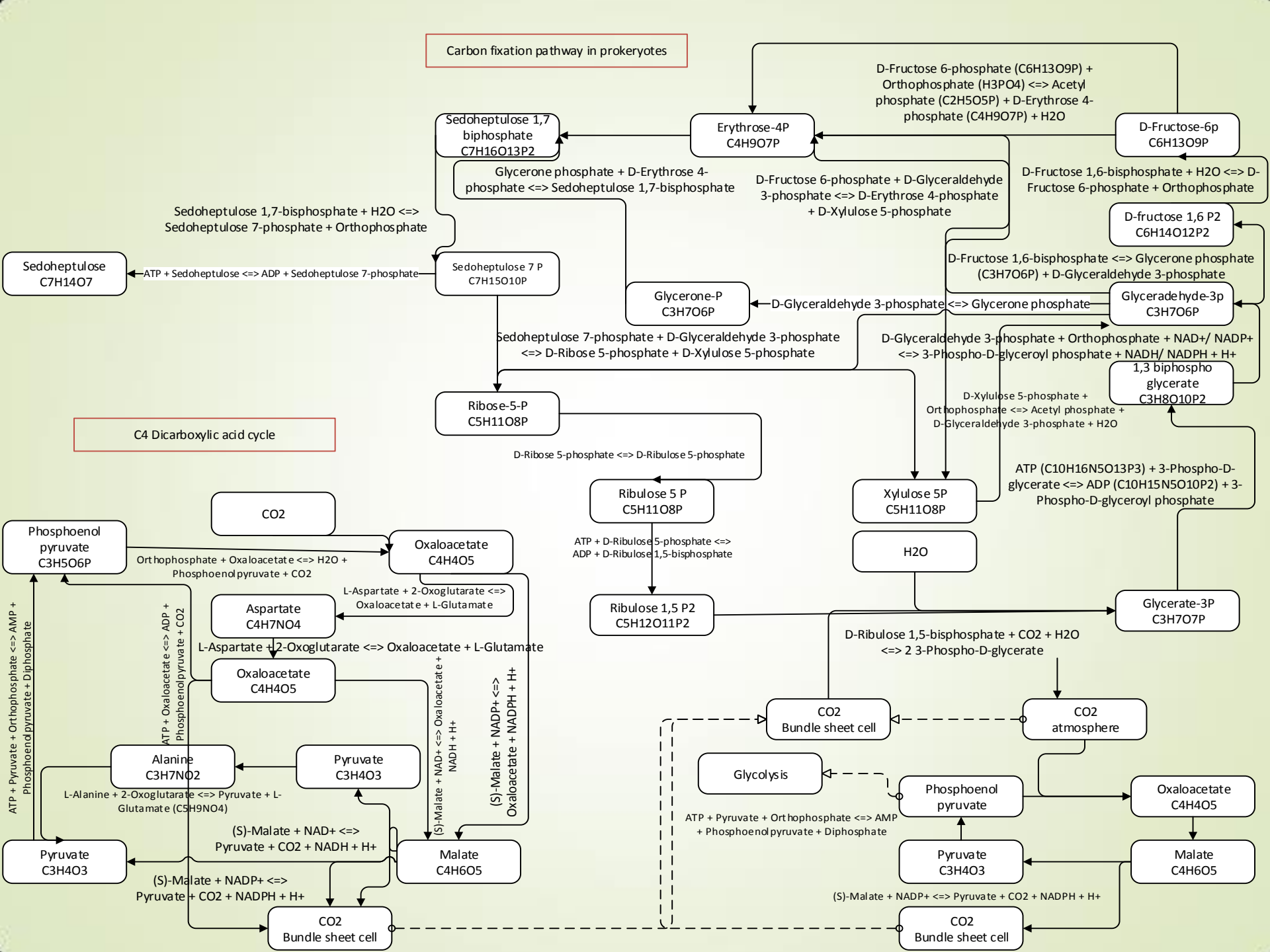
Review:

- fermentation of glucose(Saccharomyces cerevisiae)(Rizzi et al (1996)): many extracellular products such as carbon dioxide, glycerol, ethanol and acetic acid.
- fermentation of glucose(Saccharomyces cerevisiae)(Çakir et al (2004)): containing 78 reactions.
- fermentation of glucose(Saccharomyces cerevisiae)(Shi & Shimizu (1997)) and (Feng (2013)).
- fermentation of glucose (Zymomonas mobilis) (Altintas et al (2006)): Consisted of 23 equations and focused solely on the fermentation of glucose (Ethanol was the only extracellular product formed).
- fermentation of glucose (Zymomonas mobilis)(agrawal et al. (2011)).
- fermentation of glucose (thermophillic microorganisms) (Taylor et. al. (2009): the pathway of them are totally different with mesophilic.

Metabolic pathway of ethanol from glucose by s.cerevisiae




Citrate cycle
TCA cycle
S.cerevisiae
Acetyl-CoA
C21H36N7O16P3S
Oxaloacetate
C4H4O5
Citrate+ CoA (C21H36N7O16P3S) <=> Acetyl-CoA+ H2O+ Oxaloacetate
Citrate
C6H8O7
(S)-Malate + NAD+ (C21H28N7O14P2) <=>
Oxaloacetate + NADH (C21H29N7O14P2)+ H+
Citrate <=> cis-Aconitate + H2O
(S)-Malate
C4H6O5
Cis-Aconitate
C6H6O6
cis-Aconitate + H2O <=> Isocitrate
(S)-Malate <=> Fumarate + H2O
Isocitrate
C6H8O7
Isocitrate + NADP+ (C21H29N7O17P3) <=>
Oxalosuccinate + NADPH (C21H30N7O17P3) + H+
Isocitrate + NAD+ (C21H28N7O14P2) <=> 2-
Oxoglutarate + CO2 + NADH (C21H29N7O14P2) + H+
Oxalosuccinate
C6H6O7
Fumarate
C4H4O4
Oxalosuccinate <=> 2-Oxoglutarate + CO2
ThPP
Quinone (including p-Benzoquinone, o-Benzoquinone and related
compounds) + Succinate <=> Hydroquinone (C6H6O2) + Fumarate
2-Oxo-glutarate
C5H6O5
3-Carboxy-1-hydroxypropyl-ThPP + Enzyme N6-(lipoyl)lysine
(C8H14NOS2R) <=> S-succinyldihydrolipoyllysine + Thiamin
diphosphate (C12H19N4O7P2S)
IDP (C10H14N4O11P2) + Orthophosphate (H3PO4) + Succinyl-CoA
<=> ITP (C10H15N4O14P3) + Succinate + CoA (C21H36N7O16P3S)
2-Oxoglutarate + Thiamin diphosphate
(C12H19N4O7P2S) <=> 3-Carboxy-1-
hydroxypropyl-ThPP + CO2
Succinate
C4H6O4
Succinyl-CoA
C25H40N7O19P3S
S-Succinyl-
dihydrolipoamide-E
C12H20NO4S2R
3 carboxy-1-
hydroxypropyl-ThPP
C16H25N4O10P2S
Succinyl-CoA + Enzyme N6-(dihydrolipoyl)lysine <=>
CoA (C21H36N7O16P3S) + S-succinyldihydrolipoyllysine
Dihydro-lipoamide-E
C8H16NOS2R
Lipoamide-E
C8H14NOS2R
Enzyme N6-(dihydrolipoyl)lysine + NAD+ (C21H28N7O14P2)
<=> Enzyme N6-(lipoyl)lysine + NADH (C21H29N7O14P2) + H+


Carbon fixation pathway in prokeryotes
D-Fructose 6-phosphate (C6H13O9P) + Orthophosphate (H3PO4) <=> Acetyl phosphate (C2H5O5P) + D-Erythrose 4-phosphate (C4H9O7P) + H2O
Sedoheptulose 1,7 biphosphate C7H16O13P2
Erythrose-4P C4H9O7P
D-Fructose-6p C6H13O9P
Glycerone phosphate + D-Erythrose 4-phosphate <=> Sedoheptulose 1,7-bisphosphate
D-Fructose 6-phosphate + D-Glyceraldehyde 3-phosphate <=> D-Erythrose 4-phosphate + D-Xylulose 5-phosphate
D-Fructose 1,6-bisphosphate + H2O <=> D-Fructose 6-phosphate + Orthophosphate
Sedoheptulose 1,7-bisphosphate + H2O <=> Sedoheptulose 7-phosphate + Orthophosphate
D-fructose 1,6 P2 C6H14O12P2
Sedoheptulose C7H14O7
ATP + Sedoheptulose <=> ADP + Sedoheptulose 7-phosphate
Sedoheptulose 7 P C7H15O10P
D-Fructose 1,6-bisphosphate <=> Glycerone phosphate (C3H7O6P) + D-Glyceraldehyde 3-phosphate
Glycerone-P C3H7O6P
D-Glyceraldehyde 3-phosphate <=> Glycerone phosphate
Glyceradehyde-3p C3H7O6P
Sedoheptulose 7-phosphate + D-Glyceraldehyde 3-phosphate <=> D-Ribose 5-phosphate + D-Xylulose 5-phosphate
D-Glyceraldehyde 3-phosphate + Orthophosphate + NAD+/ NADP+ <=> 3-Phospho-D-glyceroyl phosphate + NADH/ NADPH + H+
1,3 biphospho glycerate C3H8O10P2
Ribose-5-P C5H11O8P
D-Xylulose 5-phosphate + Orthophosphate <=> Acetyl phosphate + D-Glyceraldehyde 3-phosphate + H2O
C4 Dicarboxylic acid cycle
D-Ribose 5-phosphate <=> D-Ribulose 5-phosphate
ATP (C10H16N5O13P3) + 3-Phospho-D-glycerate <=> ADP (C10H15N5O10P2) + 3-Phospho-D-glyceroyl phosphate
CO2
Ribulose 5 P C5H11O8P
Xylulose 5P C5H11O8P
Phosphoenol pyruvate C3H5O6P
Orthophosphate + Oxaloacetate <=> H2O + Phosphoenolpyruvate + CO2
Oxaloacetate C4H4O5
ATP + D-Ribulose 5-phosphate <=> ADP + D-Ribulose 1,5-bisphosphate
H2O
L-Aspartate + 2-Oxoglutarate <=> Oxaloacetate + L-Glutamate
Aspartate C4H7NO4
Ribulose 1,5 P2 C5H12O11P2
Glycerate-3P C3H7O7P
L-Aspartate + 2-Oxoglutarate <=> Oxaloacetate + L-Glutamate
D-Ribulose 1,5-bisphosphate + CO2 + H2O <=> 2 3-Phospho-D-glycerate
ATP + Pyruvate + Orthophosphate <=> AMP + Phosphoenolpyruvate + Diphosphate
ATP + Oxaloacetate <=> ADP + Phosphoenolpyruvate + CO2
Oxaloacetate C4H4O5
(S)-Malate + NAD+ <=> Oxaloacetate + NADH + H+
(S)-Malate + NADP+ <=> Oxaloacetate + NADPH + H+
CO2 Bundle sheet cell
CO2 atmosphere
Alanine C3H7NO2
Pyruvate C3H4O3
Glycolysis
L-Alanine + 2-Oxoglutarate <=> Pyruvate + L-Glutamate (C5H9NO4)
Phosphoenol pyruvate
Oxaloacetate C4H4O5
ATP + Pyruvate + Orthophosphate <=> AMP + Phosphoenolpyruvate + Diphosphate
(S)-Malate + NAD+ <=> Pyruvate + CO2 + NADH + H+
Pyruvate C3H4O3
Malate C4H6O5
Pyruvate C3H4O3
Malate C4H6O5
(S)-Malate + NADP+ <=> Pyruvate + CO2 + NADPH + H+
(S)-Malate + NADP+ <=> Pyruvate + CO2 + NADPH + H+
CO2 Bundle sheet cell
CO2 Bundle sheet cell

Mathematical programming

Mass balance:

For a species i being fed into a reactor the following mass balance applies:

$$\boldsymbol{F_{i,0} - F_i = \frac{\partial N_i}{\partial t} - r_i}$$

For an ideal CSTR $\partial F_i/\partial t = 0$. Converting r_i to a volume-dependent rate and substituting $\tau_{rxtr}=v/V_{rxtr}$ yields:

$$C_i = C_{i,0} + r_{i,V}\tau_{rxtr}$$

For a batch reactor the flow terms ($F_{i,0}$ and F_i) are zero, $\frac{\partial N_i}{\partial t} = r_i$. Substituting $N_i = C_i.V_{rxtr}$; applying the product rule; assuming a constant reactor volume and converting r_i to a volume-dependent rate yields:

$$\frac{\partial C_i}{\partial t} = r_{i,V}$$

Kinetic model:

The **Monod kinetic model** can be used for microbial cell biocatalyst and is described as follows:

$$\frac{S}{\mu} = \frac{K_S}{\mu_m} + \frac{S}{\mu_m}$$

In biochemistry, **Michaelis–Menten kinetics** is one of best-known models of enzyme kinetics.

$$v = \frac{d[P]}{dt} = \frac{V_{\max}[S]}{K_m + [S]}$$

Mathematical model:

This table summarizes model types that have been formulated for process engineering applications (Biegler 2010)

	LP	MILP	QP	NLP	MINLP
Process Model Building				■	■
Process Design & Synthesis Heat					
Heat Exchangers	■	■		■	■
Mass Exchangers	■	■		■	■
Separations		■		■	■
Reactors	■			■	■
Flowsheeting				■	■
Process Operations					
Scheduling	■	■			■
Supply Chain	■	■			■
Real-Time Optimization	■		■	■	
Process Control					
Model Predictive Control	■		■		
Nonlinear MPC			■	■	
Hybrid MPC		■			

GAMS (General Algebraic Modelling System):

- Used for mathematical programming (MP) and optimization.
- Used for modelling the major constraints in mathematical (linear, non-linear and mixed-integer(MILP, MINLP)) optimization problems.

MATLAB (MATrix LABoratory):

- Interpreted language for numerical computation.
- Allows one to perform numerical calculations and visualise the results without the need for complicated and time-consuming programming.

GAMS vs MATLAB:

- GAMS is unable to solve differential equations.
- MATLAB is able to solve this system due to its multitude of ODE solvers.
- Numerical approximations will be carried out in MATLAB and then transferred into GAMS in order to solve this matter.

Conclusion

- This study will model the fermenter with two different models using, firstly, Michaelis-Menten and Monod kinetics and thereafter metabolic pathway kinetics.
- Both models will be compared in order to ascertain whether the more complex models are necessary.
- It is not feasible to solve the metabolic pathway model by hand, so the computer language which will be utilised for optimisations will be Gams and Matlab.
- This approximation will be used in a mixed-integer nonlinear programming (MINLP) model to determine the optimum based on total annual cost and environmental burden.

Thank you