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Designing Predictive Mathematical Models for the Metabolic Pathways Associated with Polyhydroxybutyrate Synthesis in Escherichia coli

Angela Dixon
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DESIGNING PREDICTIVE MATHEMATICAL MODELS FOR THE METABOLIC PATHWAYS ASSOCIATED WITH POLYHYDROXYBUTYRATE SYNTHESIS IN ESCHERICHIA COLI

by

Angela Dixon

A thesis submitted in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

in

Biological Engineering

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2011
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ABSTRACT

Designing Predictive Mathematical Models for the Metabolic Pathways
Associated with Polyhydroxybutyrate Synthesis in *Escherichia coli*

by

Angela Dixon, Master of Science
Utah State University, 2011

Major Professor: Dean H. Scott Hinton
Department: Biological Engineering

Polyhydroxybutyrate (PHB) is a polyhydroxyalkanoate that has been extensively studied as a potential biodegradable replacement for petrochemically derived plastics. The synthesis pathway of PHB is native to *Ralstonia eutropha*, but the genes for the PHB pathway have successfully been introduced into *Escherichia coli* through plasmids such as the pBHR68 plasmid. However, the production of PHB needs to be more cost-effective before it can be commercially produced.

A mathematical model for PHB synthesis was developed to identify target genes that could be genetically engineered to increase PHB production. The major metabolic pathways included in the model were glycolysis, acetyl coenzyme A (acetyl-CoA) synthesis, tricarboxylic acid (TCA) cycle, glyoxylate bypass, and PHB synthesis. Each reaction in the selected metabolic pathways was modeled using the kinetic mechanism identified for the associated enzyme. The promoters and transcription factors for each enzyme were incorporated into the model. The model was validated through comparison with other published models and experimental PHB production data.
The predictive model identified 16 enzymes as having no effect on PHB production, 5 enzymes with a slight effect on PHB production, and 9 enzymes with large effects on PHB production. Decreasing the substrate affinity of the enzyme citrate synthase resulted in the largest increase in PHB synthesis. The second largest increase was observed from lowering the substrate affinity of glyceraldehyde-3-phosphate dehydrogenase. The predictive model also indicated that increasing the activity of the lac promoter in the pBHR68 plasmid resulted in the largest increase in the rate of PHB production.

The predictive model successfully identified two genes and one promoter as targets for genetic engineering to create an optimized strain of E. coli for PHB production. The substrate-binding sites for the genes gltA (citrate synthase) and gapA (glyceraldehyde-3-phosphate dehydrogenase) should be genetically engineered to be less effective at binding the substrates. The lac promoter in the pBHR68 plasmid should be genetically engineered to more closely match the consensus sequence for binding to RNA polymerase. The model predicts that an optimized strain of E. coli for PHB production could be achieved by genetically altering gltA, gapA, and the lac promoter.
Designing Predictive Mathematical Models for the Metabolic Pathways
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Plastics are a versatile and widely used material. However, traditional plastics are derived from petrochemicals and are not biodegradable. Polymers synthesized from microorganisms that have similar properties to plastic are potential biodegradable replacements. The objective of this project is to use mathematical modeling as a tool to engineer a strain of bacteria optimized for the production of bio-plastics.

Production costs can be reduced by using a bacterial strain specifically optimized for bio-plastic production. By reducing production costs, bio-plastics will be able to commercially compete with traditional plastics. Society will benefit as bio-plastics replace traditional plastics. Fossil fuels will not be depleted by the production of traditional plastics, and the bio-plastics will biodegrade in landfills.

The costs of this research are nominal. Developing a model takes only time and minimal laboratory work. An effective predictive model will reduce laboratory time and cost because it will indicate how to efficiently engineer a microorganism strain optimized for the production of bio-plastics. This design process can also be used to develop predictive models for the production of other bioproducts such as biofuels, biomaterials, and biopharmaceuticals.
ACKNOWLEDGMENTS

I am most grateful for the support of my husband, James. I could not have completed this project without his constant encouragement. I would like to thank Dean H. Scott Hinton and Dr. Ronald C. Sims for providing the opportunity to work on this research. I am grateful for their guidance and involvement in this project. I would like to thank Jia Zeng for the many hours he spent on the LC-MS for me. I am also grateful to Dr. Charles Miller and Dr. Jixun Zhan for allowing me to use their laboratories and equipment. Lastly, I would like to thank the USTAR Synthetic Bio-Manufacturing Center for funding this project.

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<td>16</td>
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INTRODUCTION

Need for Study

Plastic is one of the most heavily used compounds in the United States. Traditional plastics are versatile in use and have low production costs. However, they are produced from non-renewable petrochemicals that are not biodegradable (Reddy et al., 2003). A sustainable alternative is needed in order to decrease both the use of non-renewable fossil fuels and the buildup of plastic in landfills (Reddy et al., 2003).

Polyhydroxybutyrate (PHB) is a polyhydroxyalkanoate that has been extensively studied as a potential biodegradable replacement for petrochemical plastics (Byrom, 1987). Polyhydroxyalkanoates are storage material for many gram-positive and gram-negative bacteria (Choi & Lee, 1999). The bio-plastic is created and stored intracellularly as a reserve of carbon, energy, and reducing power during periods of stress or nutrient limitation (Doi, 1990; Lee, 1996; Madison & Huisman, 1999). The polymerization of the soluble nutrients into insoluble compounds prevents leakage out of the cell. This process allows the bacteria to have continued access to nutrients at a low maintenance cost.

Many bacteria, such as Zoogloea ramigera (Madison & Huisman, 1999; Ploux et al., 1988) and Ralstonia eutropha (Haywood et al., 1988; Haywood et al., 1989; Madison & Huisman, 1999; Slater et al., 1998; Steinbüchel & Schlegel, 1991), are capable of producing and degrading PHB and other polyhydroxyalkanoates. Genes for PHB synthesis can be cloned into other bacteria such as Escherichia coli. One of the advantages of using E. coli for the production of PHB is because E. coli is unable to degrade PHB into soluble compounds (Madison & Huisman, 1999).
The production of PHB, or any polyhydroxyalkanoate, is costly due to its carbon source and downstream processing costs (Gurieff & Lant, 2007). The high cost of $2.65-5/kg is the main reason for the limited application of these bio-plastics (Choi & Lee, 1997; Choi & Lee, 1999). Further studies are needed to help make PHB production more cost-effective before it can be commercially produced.

Metabolic modeling is a tool that has been used to determine rate-limiting steps and conditions of PHB production. However, metabolic models have focused solely on the three enzymes in the PHB synthesis pathway and have neglected to account for other key pathways in the cell (Leaf & Srienc, 1998; Van Wegen et al, 1998; Van Wegen et al, 2001). Models have also failed to account for the transcriptional network that is essential in understanding how enzymes of interest are regulated. Most models focus on optimizing the culturing methods to improve PHB production, but seldom address the possibility of optimizing the prokaryotic strain (Choi & Lee, 1997; Choi & Lee, 1999; Jurasek & Marchessault, 2004; Leaf & Srienc, 1998; Mantzaris et al, 2002; Shang et al, 2007; Van Wegen et al, 1998; Van Wegen et al, 2001).

A predictive model of the metabolic pathways of the cell should be able to quantitatively predict how changes in one pathway can affect the synthesis rate of a product of interest. This approach would require the predictive model to account for the complex metabolic pathways present in a cell. A model that focuses on one metabolic pathway lacks valuable information on what is happening elsewhere in the cell. A more complex model that accounts for many metabolic pathways and their transcriptional networks could be used as a guide in the laboratory for engineering optimized prokaryotic factories. Utilizing the synthetic biology toolbox, a strain of *E. coli* with its
metabolic pathways optimized for the synthesis of a single product could be engineered. The predictive model will allow one to target specific enzymes, promoters, or transcription factors out of many metabolic pathways for genetic engineering.

A model encompassing the major metabolic pathways associated with PHB synthesis will help determine the amount of energy and reducing power that can be diverted into the PHB synthesis pathway for the optimal PHB production. The major pathways involved in PHB synthesis that are included in the model are glycolysis, acetyl coenzyme A (acetyl-CoA) synthesis, tricarboxylic acid cycle (TCA), glyoxylate shunt, and PHB synthesis (Madison & Huisman, 1999). The model must effectively simulate the individual reactions in the previously mentioned pathways. Kinetic mechanisms, kinetic parameters, genes, and transcription factors must be determined for each reaction. Events and rules can simulate conditions that trigger the regulation of each enzyme to simulate *in vivo* conditions.

Flux balance analysis (FBA) is a tool currently used to model genome-scale metabolic networks. FBA is a constraint approach that focuses on stoichiometric information rather than kinetic data. Stoichiometric data is more widely available than kinetic data for different reactions and organisms. However, the kinetic data needed to build a genome-scale mechanistic model will become available as technology advances the collection of high throughput metabolomic data (Jamshidi & Palsson, 2008). Models based on FBA can be used to predict outcomes of gene deletion or addition, but not gene modification (Feist *et al*, 2009; Oberhardt *et al*, 2009; Orth *et al*, 2010; Price *et al*, 2003; Raman & Chandra, 2009; Schellenberger *et al*, 2011). A mechanistic model based on
kinetics could be used to predict how to genetically modify an existing enzyme to increase production of a specific bioproduct.

A constraint based approach such as FBA results in a static model whereas a kinetic based approach produces a dynamic model. As a static model, FBA also does not account for the transcriptional network that regulates enzyme expression. Attempts have been made to incorporate a regulatory network into FBA through the use of Boolean logic operators (Orth et al, 2010; Price et al, 2003; Raman & Chandra, 2009). However, a binary system based on Boolean logic operators fails to account for the entire dynamic range of the regulatory system.

Metabolic profiling is a tool capable of providing the details needed for a predictive mechanistic model. Metabolic profiling is a quantitative analysis of specific metabolites over time. The general protocol for metabolic profiling is to take samples from a culture and quench the metabolic activity. The two most common quenching methods are freezing the samples with liquid nitrogen, or shocking with a cold-buffered aqueous methanol mixture (Bajad et al, 2006; Buchholz et al, 2001; Dettmer et al, 2006; Lu et al, 2008; Mashego et al, 2007; Ohashi et al, 2007).

Once samples have been prepared, specific metabolites can be analyzed through combinations of liquid chromatography (LC), gas chromatography (GC), mass spectrometry (MS), capillary electrophoresis (CE), and nuclear magnetic resonance (NMR) (Bajad et al, 2006; Buchholz et al, 2001; Dettmer et al, 2006; Lu et al, 2008; Mashego et al, 2007; Ohashi et al, 2007). Liquid chromatography coupled with mass spectrometry (LC-MS) is a powerful tool because it is capable of analyzing many
metabolites simultaneously using a small sample volume. One study successfully analyzed as many as 69 metabolites simultaneously (Bajad et al., 2006).

LC-MS analysis can provide concentrations of metabolites at specific times in the culture when compared to standard curves. These values can then be incorporated into the predictive model so that the model more accurately represents the specific prokaryotic strain under analysis. The predictive model can then be optimized for PHB production and can identify the target genes for genetic engineering. After the prokaryotic strain has been genetically engineered, another round of metabolic profiling can be conducted to determine how the new strain performs compared to the optimized model.

Objectives

The objective of this project was to develop a mathematical model for PHB synthesis in *E. coli* for the identification of key regulators and optimal conditions for PHB synthesis. To achieve this objective, the model included pathways for glycolysis, acetyl-CoA synthesis, TCA cycle, the glyoxylate pathway, and PHB synthesis. The model incorporated enzymes, cofactors, and transcription factors for each reaction in the pathways. The model was fitted to published models and to real-time data in order to increase its accuracy. Metabolic profiling was employed to collect real-time data utilizing LC-MS for metabolite identification and quantification. Sensitivity analysis was used on the model to identify the key regulators and optimal conditions for the best PHB production. The goal of this project was to provide direction for how to genetically engineer *E. coli* to achieve higher production rates of PHB.
PATHWAYS AND REACTIONS

Metabolic pathways are composed of biochemical reactions that allow organisms to both degrade and construct compounds like PHB. Five metabolic pathways were selected to be incorporated into this mathematical model based on criteria that include energy, reducing power, and common substrates with the PHB synthesis pathway. The five metabolic pathways were PHB synthesis, TCA cycle, glyoxylate pathway, glycolysis, and acetyl-CoA synthesis.

Each pathway is a set of reactions that was modeled based on each reaction’s kinetic mechanism. The kinetic mechanism and parameters are dependent upon the enzyme that catalyzes each reaction. Studies on these enzymes have revealed many of their kinetic mechanisms and parameters. This chapter explains the justifications behind every equation and parameter used to model each reaction.

PHB Synthesis

The PHB synthesis pathway is composed of three biochemical reactions that convert acetyl-CoA into PHB. This pathway contains two intermediates: acetoacetyl-CoA and 3-hydroxybutryl-CoA. The cofactors CoA and NADPH/NADP⁺ contribute to these reactions. The enzymes β-ketothiolase (PhaA), acetoacetyl-CoA reductase (PhaB), and poly-β-hydroxybutyrate polymerase (PhaC) each catalyze one of the three reactions shown in Figure 1.
Figure 1. The metabolic pathway for the synthesis of PHB.

**Reaction 1:**

\[ 2 \text{ Acetyl-CoA} \overset{\beta\text{-ketothiolase}}{\rightarrow} \text{Acetoacetyl-CoA} + \text{CoA} \]

*Enzyme: \( \beta \)-ketothiolase*

The first step in PHB synthesis is catalyzed by the enzyme \( \beta \)-ketothiolase, also known as acetyl-CoA acetyltransferase. The enzyme \( \beta \)-ketothiolase condenses two acetyl-CoA molecules into one acetoacetyl-CoA in the forward reaction. The reversible reaction is known as the thiolysis reaction. In many organisms, such as *Ralstonia eutropha* (also previously known as *Alcaligenes eutrophus* and *Cupriavidus necator*), there are two \( \beta \)-ketothiolases able to synthesize PHB. Enzyme A can utilize the substrates acetoacetyl-CoA and 3-ketopentanoyl-CoA. Enzyme B can cleave a wider variety of substrates that include: acetoacetyl-CoA, 3-ketoheptanoyl-CoA, 3-ketopentanoyl-CoA, 3-ketohexanoyl-CoA, 3-ketoctanoyl-CoA, and 3-ketodecanoyl-CoA. Studies have shown that enzyme B is the primary \( \beta \)-ketothiolase utilized in PHB synthesis (Madison & Huisman, 1999; Slater *et al*, 1998).
Forward Reaction:

The enzyme β-ketothiolase exhibits normal Michaelis-Menten kinetics in the absence of CoA (Oeding & Schlegel, 1973; Senior & Dawes, 1973). In multiple organisms, the enzyme displayed competitive-inhibition in the presence of CoA (Haywood et al, 1988; Mothes et al, 1997; Oeding & Schlegel, 1973; Steinbüchel & Schlegel, 1991). Based on the non-linear Lineweaver-Burk plots in the presence of CoA, Oeding and Schlegel proposed a ping-pong mechanism with CoA as the binary term. Hill kinetics were observed with coefficients of n = 1 in the absence of CoA and n = 2 in the presence of CoA (Oeding & Schlegel, 1973).

Based on the findings described above, it was decided to use Equation (1) for the condensation reaction. Equation (1) combines the kinetics of competitive-inhibition with Hill cooperativity. Acetyl-CoA was used as the substrate, S, and CoA was used as the inhibitor, I. The kinetic parameters utilized in simulations of this model are shown in Table I. No kinetic data were available on E. coli for β-ketothiolase. Because the genes for β-ketothiolase in E. coli come from R. eutropha, when available, it was preferable to use kinetic data from R. eutropha (or A. eutrophus, C. necator) rather than from other organisms. Because the enzyme B of β-ketothiolase is preferred over the enzyme A in PHB synthesis (Madison & Huisman, 1999; Slater et al, 1998), $K_m$ values for enzyme B were chosen over enzyme A in this model. No value in the literature was found for $V_m$ in R. eutropha. All values found for $V_m$ are shown in Table I.

Reverse Reaction:

In the direction of thiolysis, β-ketothiolase does not obey Michaelis-Menten kinetics. Studies indicate that there is a positive cooperativity between acetoacetyl-CoA
and CoA (Haywood et al., 1988; Steinbüchel & Schlegel, 1991). Lineweaver-Burk plots yield a family of parallel lines at varied concentrations of acetoacetyl-CoA or CoA. Parallel lines indicate that this reaction follows a ping-pong bi-bi mechanism (Molina et al., 1994; Oeding & Schlegel, 1973).

For this model, it was decided to use Equation (2), the ping-pong bi-bi mechanism, for the thiolysis reaction. Acetoacetyl-CoA was used as substrate A, and CoA was used as substrate B. Kinetic parameters utilized in simulations of this model are shown in Table I. No kinetic data were available on *E. coli* for β-ketothiolase.

\[
\nu = \frac{V_{\text{MAX}}[S]^n}{K_M(1+\frac{I}{K_i})+[S]^n} = \frac{k_{\text{cat}}*[E]*[S]^n}{K_M(1+\frac{I}{K_i})+[S]^n}
\]

(1)

\[
V_{\text{MAX}} = k_{\text{cat}}*[E] = [\mu M/\text{s}]
\]

\[
K_M = \text{Substrate concentration resulting in 0.5}V_{\text{MAX}} = [\mu M]
\]

\[
K_i = \text{Inhibitor concentration in 0.5}V_{\text{MAX}} = [\mu M]
\]

\[
k_{\text{cat}} = \text{Maximum number of rxns catalyzed per second} = [s^{-1}]
\]

\[
[S] = \text{Concentration of substrate} = [\mu M]
\]

\[
[E] = \text{Concentration of enzyme} = [\mu M]
\]

\[
[I] = \text{Concentration of inhibitor} = [\mu M]
\]

\[
n = \text{Hill cooperativity coefficient}
\]

\[
\nu = \frac{V_{\text{MAX}}}{\frac{K_A}{[A]} + \frac{K_B}{[B]} + 1} = \frac{k_{\text{cat}}*[E]}{\frac{K_A}{[A]} + \frac{K_B}{[B]} + 1}
\]

(2)

\[
V_{\text{MAX}} = k_{\text{cat}}*[E] = [\mu M/\text{s}]
\]

\[
K_A = \text{Dissociation constant for substrate A} = [\mu M]
\]

\[
K_B = \text{Dissociation constant for substrate B} = [\mu M]
\]

\[
k_{\text{cat}} = \text{Maximum number of rxns catalyzed per second} = [s^{-1}]
\]

\[
[A] = \text{Concentration of substrate A} = [\mu M]
\]

\[
[B] = \text{Concentration of substrate B} = [\mu M]
\]

\[
[E] = \text{Concentration of enzyme} = [\mu M]
\]
Table I. Kinetic parameters utilized for Reaction 1 in the model.

<table>
<thead>
<tr>
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<th>Units</th>
<th>Organism</th>
<th>Reference</th>
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<tbody>
<tr>
<td>$K_m$</td>
<td>230$^b$</td>
<td>$\mu M$</td>
<td><em>A. eutrophus</em></td>
<td>Steinbüchel &amp; Schlegel, 1991</td>
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<td></td>
<td>390</td>
<td>$\mu M$</td>
<td><em>C. necator</em></td>
<td>Oeding &amp; Schlegel, 1973</td>
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<tr>
<td></td>
<td>1100$^a$</td>
<td>$\mu M$</td>
<td><em>A. eutrophus</em></td>
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<td>$V_m$</td>
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<td>$K_m$</td>
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<td>0.0177</td>
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<td>0.32</td>
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<td>$K_i$</td>
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<td>$n$</td>
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<td>-</td>
<td><em>A. eutrophus</em></td>
<td>Steinbüchel &amp; Schlegel, 1991</td>
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<table>
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<th>Value</th>
<th>Units</th>
<th>Organism</th>
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<td></td>
<td>394$^b$</td>
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<tr>
<td>$K_B$</td>
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</tr>
<tr>
<td></td>
<td>93$^b$</td>
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<td><em>A. eutrophus</em></td>
<td>Steinbüchel &amp; Schlegel, 1991</td>
</tr>
<tr>
<td>$V_m$</td>
<td>1.24</td>
<td>$\mu M/s$</td>
<td><em>M. rhodesianum</em></td>
<td>Mothes <em>et al.</em>, 1997</td>
</tr>
</tbody>
</table>

$^a$. Enzyme A of β-ketothiolase
$^b$. Enzyme B of β-ketothiolase

**Reaction 2:**

\[
\text{Acetoacetyl-CoA + NADPH + H}^+ \overset{\text{Acetoacetyl-CoA reductase}}{\rightleftharpoons} \text{3-hydroxybutyryl-CoA + NADP}^+ 
\]

**Enzyme: Acetoacetyl-CoA reductase**

Acetoacetyl-CoA reductase catalyzes the second step in PHB synthesis by converting acetoacetyl-CoA into 3-hydroxybutyryl-CoA. It has been classified as a NADPH-dependent reductase. The availability of reducing power is one of the main driving forces for PHB synthesis. Thiolysis is the thermodynamically favored direction; however, under favorable PHB accumulating conditions, acetoacetyl-CoA reductase can pull the reaction in the condensation direction (Madison & Huisman, 1999).
**Forward Reaction:**

The kinetics of acetoacetyl-CoA reductase vary by organism. The enzyme has been shown to have cooperativity factors in *M. extorquens* for acetoacetyl-CoA and NADPH (Belova et al., 2006). However, it has been shown that there are no cooperativity factors in *M. rhodesianum* (Mothes & Babel, 1994). Kinetic behavior indicates inhibition with the substrate acetoacetyl-CoA at high concentrations in multiple organisms (Belova et al., 2006; Mothes & Babel, 1994; Ploux et al., 1988). Normal Michaelis-Menten kinetics have been observed in *A. beijerinckii* (Ritchie et al., 1971). Also, a sequential kinetic mechanism has been observed in *Z. ramigera* (Ploux et al., 1988).

The literature contained *Kₘ* values for acetoacetyl-CoA and NADPH/NADH (Steinbüchel & Schlegel, 1991) in *A. eutrophus*, but no mechanism was proposed. Due to the dependence on NADPH/NADH and the sequential kinetic mechanism observed in *Z. ramigera*, it was decided to use an ordered bi-bi mechanism, Equation (3), to model this reaction. Substrate A was acetoacetyl-CoA, and substrate B was NADPH/NADH. The NADPH dependent enzyme is the one used in PHB synthesis (Madison & Huisman, 1999); therefore, kinetic parameters for the NADPH dependent enzyme shown in Table II were used in the model.

\[
V = \frac{V_{MAX}}{\frac{K_A K_B}{[A][B]} + \frac{K_A}{[A]} + \frac{K_B}{[B]} + 1} = \frac{K_{cat} \cdot [E]}{\frac{K_A K_B}{[A][B]} + \frac{K_A}{[A]} + \frac{K_B}{[B]} + 1}
\]

\[
V_{MAX} = k_{cat} \cdot [E] = [\mu M/s]
\]

\[
K_A = \text{Dissociation constant for substrate A} = [\mu M]
\]

\[
K_B = \text{Dissociation constant for substrate B} = [\mu M]
\]

\[
k_{cat} = \text{Maximum number of rxns catalyzed per second} = [s^{-1}]
\]

\[
[A] = \text{Concentration of substrate A} = [\mu M]
\]

\[
[B] = \text{Concentration of substrate B} = [\mu M]
\]

\[
[E] = \text{Concentration of enzyme} = [\mu M]
\]
Reverse Reaction:

The literature contained no proposed kinetic mechanism for the thiolysis reaction catalyzed by acetoacetyl-CoA reductase. However, $K_m$ values for β-hydroxybutyryl-CoA and NADP⁺/NAD⁺ were found in *A. eutrophus* (Steinbüchel & Schlegel, 1991). It was decided to model this reaction with the same mechanism utilized in the condensation reaction. An ordered bi-bi mechanism, Equation (3), was employed with substrate A as β-hydroxybutyryl-CoA and substrate B as NADP⁺/NAD⁺.

Although both NADPH and NADH dependent acetoacetyl-CoA reductase exist in *A. eutrophus*, only the NADPH dependent enzyme is used in PHB synthesis (Madison & Huisman, 1999). Therefore, kinetic parameters for the NADPH dependent enzyme were used in the model as shown in Table II. The parameter $V_m$ was not found for the thiolysis reaction. The same value used in the condensation reaction was used in the thiolysis reaction model.

**Table II. Kinetic parameters utilized for Reaction 2 in the model.**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Units</th>
<th>Organism</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fwd</td>
<td>$K_A$</td>
<td>5$^a$</td>
<td>μM</td>
<td><em>A. eutrophus</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td>22$^b$</td>
<td>μM</td>
<td><em>A. eutrophus</em></td>
</tr>
<tr>
<td></td>
<td>$K_B$</td>
<td>19$^a$</td>
<td>μM</td>
<td><em>A. eutrophus</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td>13$^b$</td>
<td>μM</td>
<td><em>A. eutrophus</em></td>
</tr>
<tr>
<td></td>
<td>$V_m$</td>
<td>0.023</td>
<td>μM/s</td>
<td><em>R. sphaeroides</em></td>
</tr>
<tr>
<td>Rev</td>
<td>$K_A$</td>
<td>33$^a$</td>
<td>μM</td>
<td><em>A. eutrophus</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td>26$^b$</td>
<td>μM</td>
<td><em>A. eutrophus</em></td>
</tr>
<tr>
<td></td>
<td>$K_B$</td>
<td>31$^a$</td>
<td>μM</td>
<td><em>A. eutrophus</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td>16$^b$</td>
<td>μM</td>
<td><em>A. eutrophus</em></td>
</tr>
<tr>
<td></td>
<td>$V_m$</td>
<td>0.023</td>
<td>μM/s</td>
<td><em>R. sphaeroides</em></td>
</tr>
</tbody>
</table>

*a.* NADPH dependent

*b.* NADH dependent
Reaction 3:

\[(\text{n})3\text{-hydroxybutyryl-CoA} \xrightarrow{\text{PHB polymerase}} \text{poly-3-hydroxybutyrate} + (\text{n})\text{CoA}\]

**Enzyme: Poly-β-hydroxybutyrate polymerase (PHB synthase)**

The enzyme poly-β-hydroxybutyrate polymerase, also known as PHB synthase, catalyzes the third step in the PHB synthesis pathway. This enzyme exists in both soluble and granule-associated forms (Haywood *et al.*, 1989; Madison & Huisman, 1999). In carbon-limited environments, most PHB synthase is soluble. In nitrogen-limited environments, the majority of PHB synthase is granule-associated (Haywood *et al.*, 1989). PHB synthase can polymerize 3-hydroxybutyrate units to form poly-3-hydroxybutyrate (PHB) and 3-hydroxyvalerate units to form polyhydroxyalkanoate (PHA) (Haywood *et al.*, 1989).

**Forward Reaction:**

Poly-β-hydroxybutyrate polymerase demonstrated normal Michaelis-Menten kinetics in *A. eutrophus* (Haywood *et al.*, 1989). Equation (4) was used to model the forward reaction using kinetic parameters from Table III. The molecular weight of PHB assumed for this model was 160,000 Daltons because of its association with the kinetic parameters in Table III (Haywood *et al.*, 1989). Using the molecular weight of 160 kDa results in a PHB polymer composed of 1,860 units of 3-hydroxybutyrate. The stoichiometric coefficient \(n\) in Reaction 3 was set as 1,860 in the model. Kinetic parameters for the granule-associated PHB synthase were used in the model due to the unstable nature of the soluble PHB synthase (Haywood *et al.*, 1989).
Reverse Reaction:

The reverse reaction was ignored in this model because *E. coli* do not naturally code for PHB depolymerase (Saito *et al.*, 1989). The PHB depolymerase gene will not be cloned into *E. coli* because it is desired to accumulate, not degrade, PHB.

\[
V = \frac{V_{\text{MAX}}[S]}{K_M + [S]} = \frac{k_{\text{cat}}[E][S]}{K_M + [S]}
\]

(4)

- \( V_{\text{MAX}} = k_{\text{cat}}[E] = [\mu \text{M/s}] \)
- \( K_M = \) Substrate concentration resulting in \(0.5V_{\text{MAX}} = [\mu \text{M}] \)
- \( k_{\text{cat}} = \) Maximum number of rxns catalyzed per second = \([s^{-1}]\)
- \( [S] = \) Concentration of substrate = \([\mu \text{M}]\)
- \( [E] = \) Concentration of enzyme = \([\mu \text{M}]\)

Table III. Kinetic parameters utilized for Reaction 3 in the model.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Units</th>
<th>Organism</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>( K_M )</td>
<td>680^{a,c}</td>
<td>\mu \text{M}</td>
<td><em>A. eutrophus</em></td>
<td>Haywood <em>et al.</em>, 1989</td>
</tr>
<tr>
<td><strong>Fwd</strong></td>
<td>720^{a,d}</td>
<td>\mu \text{M}</td>
<td><em>A. eutrophus</em></td>
<td>Haywood <em>et al.</em>, 1989</td>
</tr>
<tr>
<td><strong>Rxn</strong></td>
<td>1630^{b,c}</td>
<td>\mu \text{M}</td>
<td><em>A. eutrophus</em></td>
<td>Haywood <em>et al.</em>, 1989</td>
</tr>
<tr>
<td>( V_m )</td>
<td>0.0047</td>
<td>\mu \text{M/s}</td>
<td><em>A. eutrophus</em></td>
<td>Haywood <em>et al.</em>, 1989</td>
</tr>
</tbody>
</table>

- a. (R)-3-hydroxybutyryl-CoA as substrate
- b. (R)-3-hydroxyvaleryl-CoA as substrate
- c. Granule associated synthase
- d. Soluble synthase

TCA Cycle

The tricarboxylic acid (TCA) cycle is composed of nine reactions that utilize acetyl-CoA to generate energy and reducing power. Nine enzymes catalyze the reactions in the TCA cycle as shown in Figure 2. The TCA cycle uses the cofactors CoA, ADP/ATP, NAD\(^+\)/NADH, and NADP\(^+\)/NADPH. The TCA cycle takes a portion of acetyl-CoA away from PHB synthesis. Cells need energy, reducing power, and acetyl-CoA to produce PHB, so the TCA cycle is an important pathway to include in the model.
Figure 2. The metabolic pathway for the TCA cycle.

Reaction 4:

\[
\text{Oxaloacetate} + \text{Acetyl-CoA} + \text{H}_2\text{O} \xleftrightarrow{\text{Citrate synthase}} \text{Citrate} + \text{CoA} + \text{H}^+ 
\]

Enzyme: Citrate synthase

Citrate synthase catalyzes the Claisen condensation reaction that forms citrate from oxaloacetate and acetyl-CoA. Citrate synthase is controlled through activators and inhibitors. Acetyl-CoA acts as an allosteric activator and K\(^+\) acts as a non-allosteric inhibitor. Allosteric inhibitors include NADH, NAD\(^+\), and oxaloacetate. ATP and 2-oxoglutarate act as competitive inhibitors. Other inhibitors include citrate, isocitrate, and cis-aconitate (Anderson & Duckworth, 1988; Duckworth et al, 1987; Man et al, 1995; Senior & Dawes, 1973; Walsh & Koshland, 1985).
**Forward Reaction:**

Studies have demonstrated sequential ordered bi-bi kinetics, with oxaloacetate binding first, in *E. coli* for this reaction (Anderson & Duckworth, 1988). Studies have also demonstrated that citrate synthase exhibits competitive inhibition with 2-oxoglutarate (Anderson & Duckworth, 1988; Pereira *et al.*, 1994).

An ordered bi-bi kinetic mechanism, Equation (3), was used to model the forward reaction catalyzed by citrate synthase. Substrate A was oxaloacetate and substrate B was acetyl-CoA. Kinetic parameters utilized in the model are shown in Table IV. The values chosen were from various strains of *E. coli*.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Units</th>
<th>Organism</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_A$</td>
<td>460</td>
<td>$\mu$M</td>
<td><em>E. coli</em> H229Q</td>
<td>Anderson &amp; Duckworth, 1988</td>
</tr>
<tr>
<td></td>
<td>17</td>
<td>$\mu$M</td>
<td><em>E. coli</em> H226Q</td>
<td>Anderson &amp; Duckworth, 1988</td>
</tr>
<tr>
<td></td>
<td>26</td>
<td>$\mu$M</td>
<td><em>E. coli</em> wild</td>
<td>Anderson &amp; Duckworth, 1988</td>
</tr>
<tr>
<td>$K_B$</td>
<td>190</td>
<td>$\mu$M</td>
<td><em>E. coli</em> H229Q</td>
<td>Anderson &amp; Duckworth, 1988</td>
</tr>
<tr>
<td></td>
<td>260</td>
<td>$\mu$M</td>
<td><em>E. coli</em> H226Q</td>
<td>Anderson &amp; Duckworth, 1988</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>$\mu$M</td>
<td><em>E. coli</em> wild</td>
<td>Anderson &amp; Duckworth, 1988</td>
</tr>
<tr>
<td>$k_{cat}$</td>
<td>9.3</td>
<td>$s^{-1}$</td>
<td><em>E. coli</em> H229Q</td>
<td>Anderson &amp; Duckworth, 1988</td>
</tr>
<tr>
<td></td>
<td>98</td>
<td>$s^{-1}$</td>
<td><em>E. coli</em> H226Q</td>
<td>Anderson &amp; Duckworth, 1988</td>
</tr>
<tr>
<td></td>
<td>81</td>
<td>$s^{-1}$</td>
<td><em>E. coli</em> wild</td>
<td>Anderson &amp; Duckworth, 1988</td>
</tr>
<tr>
<td>$K_A$</td>
<td>159</td>
<td>$\mu$M</td>
<td>Rat kidney</td>
<td>Matsuoka &amp; Srere, 1973</td>
</tr>
<tr>
<td></td>
<td>420</td>
<td>$\mu$M</td>
<td>Human heart</td>
<td>Mukherjee <em>et al.</em>, 1980</td>
</tr>
<tr>
<td>$K_B$</td>
<td>32</td>
<td>$\mu$M</td>
<td>Rat kidney</td>
<td>Matsuoka &amp; Srere, 1973</td>
</tr>
<tr>
<td></td>
<td>70</td>
<td>$\mu$M</td>
<td>Human heart</td>
<td>Mukherjee <em>et al.</em>, 1980</td>
</tr>
<tr>
<td>$V_m$</td>
<td>1.67E-4</td>
<td>$\mu$M/s</td>
<td>Rat kidney</td>
<td>Matsuoka &amp; Srere, 1973</td>
</tr>
</tbody>
</table>

**Reverse Reaction:**

No kinetic data were found for the reverse of Reaction 4 in *E. coli*. Studies of the citrate synthase in rat kidneys and the human heart provided kinetic parameters for the reverse reaction catalyzed by citrate synthase (Matsuoka & Srere, 1973; Mukherjee *et al.*, 1980).
1980). Studies suggest that a random bi-bi mechanism is employed by this reaction (Matsuoka & Srere, 1973). Equation (5) represents a random bi-bi kinetic mechanism. Therefore, Equation (5) and the kinetic parameters in Table IV were used to model the reverse reaction in SimBiology with substrate A as citrate and substrate B as CoA.

\[
V = \frac{V_{MAX}}{\frac{\alpha K_A K_B}{[A][B]} + \frac{\alpha K_A}{[A]} + \frac{\alpha K_B}{[B]} + 1} = \frac{k_{cat}^* [E]}{\frac{\alpha K_A K_B}{[A][B]} + \frac{\alpha K_A}{[A]} + \frac{\alpha K_B}{[B]} + 1}
\]

\[
V_{MAX} = k_{cat}^* [E] = [\mu M/s]
K_A = \text{Dissociation constant for substrate A} = [\mu M]
K_B = \text{Dissociation constant for substrate B} = [\mu M]
k_{cat} = \text{Maximum number of rxns catalyzed per second} = [s^{-1}]
[A] = \text{Concentration of substrate A} = [\mu M]
[B] = \text{Concentration of substrate B} = [\mu M]
[E] = \text{Concentration of enzyme} = [\mu M]
\alpha = \text{Binding interaction factor}
\text{If } \alpha = 1, \text{ substrate binding is independent}
\text{If } \alpha > 1, \text{ binding of substrate decreases affinity of other}
\text{If } \alpha < 1, \text{ binding of substrate increases affinity of other}

**Reaction 5:**

\[
\text{Citrate} \quad \text{Citrate hydro-lyase} \quad \text{Citrate hydro-lyase} \implies \text{Aconitate} + H_2O
\]

**Enzyme: Citrate hydro-lyase**

Citrate hydro-lyase is an aconitase that catalyzes the reversible isomerization of citrate. *E. coli* has two major aconitases, AcnA and AcnB. AcnA is the aerobic-stationary phase enzyme, and AcnB is the major TCA cycle enzyme during exponential growth. AcnA is more stable than AcnB, and has a higher affinity for citrate. AcnB is the main catabolic enzyme because its sensitivity to oxidative or pH stress allows it to regulate the TCA cycle (Jordan *et al*, 1999).
**Forward Reaction:**

Studies indicate that this reaction exhibits normal Michaelis-Menten kinetics. Equation (4) was used to model the forward reaction. Kinetic parameters were found for both AcnA and AcnB as seen in Table V. It was decided to use AcnB values for this model because AcnB is the major TCA cycle enzyme (Jordan et al, 1999).

**Reverse Reaction:**

Studies indicate that this reaction exhibits Michaelis-Menten kinetics with a Hill cooperativity factor of 2.1 (Tsuchiya et al, 2009). Equation (6) represents Hill kinetics and was used to model the reverse reaction with the kinetic parameters found in Table V.

\[
V = \frac{V_{\text{MAX}}[S]^n}{K_M + [S]^n} = \frac{k_{\text{cat}}*[E][S]^n}{K_M + [S]^n}
\]  

(6)

\[
V_{\text{MAX}} = k_{\text{cat}}*[E] = [\mu M/s]
\]

\[
K_M = \text{Substrate concentration resulting in } 0.5V_{\text{MAX}} = [\mu M]
\]

\[
k_{\text{cat}} = \text{Maximum number of rxns catalyzed per second } = [s^{-1}]
\]

\[
[S] = \text{Concentration of substrate } = [\mu M]
\]

\[
[E] = \text{Concentration of enzyme } = [\mu M]
\]

\[
n = \text{Hill cooperativity coefficient}
\]

Table V. Kinetic parameters utilized for Reaction 5 in the model.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Units</th>
<th>Organism</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fwd</td>
<td>K_m</td>
<td>1160^a</td>
<td>(\mu M)</td>
<td>E. coli K-12 MG1655</td>
</tr>
<tr>
<td>Rxn</td>
<td></td>
<td>11000^b</td>
<td>(\mu M)</td>
<td>E. coli K-12 MG1655</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(\mu M/s)</td>
<td>E. coli K-12 MG1655</td>
</tr>
<tr>
<td>V_m</td>
<td>0.102^a</td>
<td>0.397^b</td>
<td>(\mu M/s)</td>
<td>E. coli K-12 MG1655</td>
</tr>
<tr>
<td>Rev</td>
<td>K_m</td>
<td>58^a</td>
<td>(\mu M)</td>
<td>E. coli K-12 MG1655</td>
</tr>
<tr>
<td>Rxn</td>
<td>V_m</td>
<td>16^b</td>
<td>(\mu M)</td>
<td>E. coli K-12 MG1655</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.242^a</td>
<td>(\mu M/s)</td>
<td>E. coli K-12 MG1655</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.652^b</td>
<td>(\mu M/s)</td>
<td>E. coli K-12 MG1655</td>
</tr>
<tr>
<td>n</td>
<td>2.1</td>
<td></td>
<td></td>
<td>E. coli K-12 MG1655</td>
</tr>
</tbody>
</table>

a. acnA
b. acnB
Reaction 6:

\[ \text{Aconitate} + \text{H}_2\text{O} \xrightleftharpoons{\text{Isocitrate hydro-lyase}} \text{Isocitrate} \]

**Enzyme: Isocitrate hydro-lyase**

Isocitrate hydro-lyase is an aconitase that catalyzes the reversible isomerization of aconitate. As mentioned previously, *E. coli* has two major aconitases, AcnA and AcnB.

**Forward Reaction:**

Studies show that this reaction exhibits normal Michaelis-Menten kinetics with negative cooperativity (Jordan *et al.*, 1999; Tsuchiya *et al.*, 2009). Equation (6) was used to model the forward reaction. Kinetic parameters were found for both AcnA and AcnB as seen in Table VI. AcnB values were used for this model because AcnB is the major TCA cycle enzyme (Jordan *et al.*, 1999).

Table VI. Kinetic parameters utilized for Reaction 6 in the model.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Units</th>
<th>Organism</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fwd</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>K&lt;sub&gt;m&lt;/sub&gt;</td>
<td>58&lt;sup&gt;a&lt;/sup&gt;</td>
<td>µM</td>
<td><em>E. coli</em> K-12 MG1655</td>
<td>Jordan <em>et al.</em>, 1999</td>
</tr>
<tr>
<td></td>
<td>16&lt;sup&gt;b&lt;/sup&gt;</td>
<td>µM</td>
<td><em>E. coli</em> K-12 MG1655</td>
<td>Jordan <em>et al.</em>, 1999</td>
</tr>
<tr>
<td>V&lt;sub&gt;m&lt;/sub&gt;</td>
<td>0.242&lt;sup&gt;a&lt;/sup&gt;</td>
<td>µM/s</td>
<td><em>E. coli</em> K-12 MG1655</td>
<td>Jordan <em>et al.</em>, 1999</td>
</tr>
<tr>
<td></td>
<td>0.652&lt;sup&gt;b&lt;/sup&gt;</td>
<td>µM/s</td>
<td><em>E. coli</em> K-12 MG1655</td>
<td>Jordan <em>et al.</em>, 1999</td>
</tr>
<tr>
<td>n</td>
<td>0.727</td>
<td>-</td>
<td><em>E. coli</em> K-12 MG1655</td>
<td>Tsuchiya <em>et al.</em>, 2009</td>
</tr>
<tr>
<td><strong>Rev</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>K&lt;sub&gt;m&lt;/sub&gt;</td>
<td>14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>µM</td>
<td><em>E. coli</em> K-12 MG1655</td>
<td>Jordan <em>et al.</em>, 1999</td>
</tr>
<tr>
<td></td>
<td>51&lt;sup&gt;b&lt;/sup&gt;</td>
<td>µM</td>
<td><em>E. coli</em> K-12 MG1655</td>
<td>Jordan <em>et al.</em>, 1999</td>
</tr>
<tr>
<td>V&lt;sub&gt;m&lt;/sub&gt;</td>
<td>0.0595&lt;sup&gt;a&lt;/sup&gt;</td>
<td>µM/s</td>
<td><em>E. coli</em> K-12 MG1655</td>
<td>Jordan <em>et al.</em>, 1999</td>
</tr>
<tr>
<td></td>
<td>0.0987&lt;sup&gt;b&lt;/sup&gt;</td>
<td>µM/s</td>
<td><em>E. coli</em> K-12 MG1655</td>
<td>Jordan <em>et al.</em>, 1999</td>
</tr>
</tbody>
</table>

<sup>a</sup> acnA  
<sup>b</sup> acnB
**Reverse Reaction:**

Studies indicate that this reaction exhibits Michaelis-Menten kinetics (Jordan *et al*, 1999). Equation (4) was used to model the reverse reaction. Kinetic parameters were found for both AcnA and AcnB as seen in Table VI. AcnB values were used for this model because AcnB is the major TCA cycle enzyme (Jordan *et al*, 1999).

**Reaction 7:**

\[
\text{Isocitrate} + \text{NADP}^+ \xrightarrow{\text{Isocitrate dehydrogenase}} 2\text{-oxoglutarate} + \text{NADPH} + \text{CO}_2
\]

**Enzyme: Isocitrate dehydrogenase**

Isocitrate dehydrogenase catalyzes the irreversible oxidative decarboxylation of isocitrate to 2-oxoglutarate. The enzyme is regulated by phosphorylation and dephosphorylation. Isocitrate is important because it allows *E. coli* to switch between the TCA cycle and the glyoxylate bypass pathway.

**Forward Reaction:**

Studies indicate that the reaction catalyzed by isocitrate dehydrogenase displays normal Michaelis-Menten kinetics (Lee *et al*, 1995). Therefore, Equation (4) was used to model the reaction utilizing the kinetic parameters from Table VII.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Units</th>
<th>Organism</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_m$</td>
<td>11.4</td>
<td>µM</td>
<td><em>E. coli</em></td>
<td>Lee <em>et al</em>, 1995</td>
</tr>
<tr>
<td></td>
<td>6030</td>
<td>µM</td>
<td><em>E. coli</em> K230M</td>
<td>Lee <em>et al</em>, 1995</td>
</tr>
<tr>
<td>$k_{cat}$</td>
<td>9.6</td>
<td>µM</td>
<td><em>E. coli</em> Y160F</td>
<td>Lee <em>et al</em>, 1995</td>
</tr>
<tr>
<td><strong>Fwd</strong></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Rxn</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>$k_{cat}$</td>
<td>76.2</td>
<td>s$^{-1}$</td>
<td><em>E. coli</em></td>
<td>Lee <em>et al</em>, 1995</td>
</tr>
<tr>
<td></td>
<td>0.85</td>
<td>s$^{-1}$</td>
<td><em>E. coli</em> K230M</td>
<td>Lee <em>et al</em>, 1995</td>
</tr>
<tr>
<td></td>
<td>0.311</td>
<td>s$^{-1}$</td>
<td><em>E. coli</em> Y160F</td>
<td>Lee <em>et al</em>, 1995</td>
</tr>
</tbody>
</table>
**Reaction 8:**

\[
2\text{-oxoglutarate} + \text{CoA} + \text{NAD}^+ \xrightleftharpoons{2\text{-oxoglutarate dehydrogenase}} \text{Succinyl-CoA} + \text{NADH} + \text{CO}_2
\]

*Enzyme: 2-oxoglutarate dehydrogenase complex*

The 2-oxoglutarate dehydrogenase complex catalyzes the irreversible oxidative decarboxylation of 2-oxoglutarate to succinyl-CoA. The complex contains three components: 2-oxoglutarate decarboxylase (E1), lipoamide acyltransferase (E2), and lipoamide dehydrogenase (E3).

*Forward Reaction:*

The net forward reaction demonstrated normal Michaelis-Menten kinetics (McCormack & Denton, 1981). The kinetic parameters in Table VIII were used with Equation (4) to model the forward reaction.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Units</th>
<th>Organism</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>K(_m) Fwd Rxn</td>
<td>9.2</td>
<td>µM</td>
<td><em>E. coli</em></td>
<td>McCormack &amp; Denton, 1981</td>
</tr>
<tr>
<td>V(_m) Fwd Rxn</td>
<td>10.5</td>
<td>µM</td>
<td><em>E. coli</em></td>
<td>McCormack &amp; Denton, 1981</td>
</tr>
<tr>
<td>V(_m) Fwd Rxn</td>
<td>0.00363</td>
<td>µM /s</td>
<td><em>E. coli</em></td>
<td>McCormack &amp; Denton, 1981</td>
</tr>
</tbody>
</table>

**Reaction 9:**

\[
\text{Succinyl-CoA} + \text{ADP} + \text{P}^i + \text{H}^+ \xrightleftharpoons{\text{Succinyl-CoA synthetase}} \text{Succinate} + \text{ATP} + \text{CoA}
\]

*Enzyme: Succinyl-CoA synthetase*

Succinyl-CoA synthetase catalyzes substrate level phosphorylation in the TCA cycle by converting succinyl-CoA and ADP into succinate and ATP. In *E. coli*, the
enzyme exists as a tetramer and favors adenine over guanine nucleotides. In eukaryotes, guanine nucleotides are preferred (Birney et al, 1996). It is a multiple step reaction that uses a covalent enzyme-substrate intermediate. The phosphate group is transferred to the enzyme resulting in a phosphoenzyme that is then used to convert ADP into ATP.

_Forward Reaction:_

In the forward reaction, enzyme bound succinyl-phosphate is formed as an intermediate. The phosphate group is transferred to a histidine residue and succinate is released as a product. The phosphoenzyme then generates the second product, ATP.

The kinetic mechanism behind this reaction is not well understood. The reaction is similar to many reactions classified as ping-pong, but some studies support a sequential mechanism and the formation of a quaternary structure. Studies indicate that ADP is an allosteric regulator of the enzyme during the forward reaction (Um & Klein, 1993). The reaction displays an unusual catalytic property called substrate synergism. The presence of a substrate for one reaction stimulates another reaction. The exact mechanism for substrate synergism has yet to be defined in terms of an equation (Birney et al, 1996; Um & Klein, 1993).

Due to the lack of a kinetic mechanism and parameters, this reaction was modeled by using simple mass-action kinetics. It is hoped that future studies can provide a more accurate kinetic mechanism for this specific reaction.

_Reverse Reaction:_

The reverse reaction also has unusual catalytic properties, but some studies supported a sequential mechanism and were able to measure some kinetic parameters.
(Joyce et al., 1999; Luo & Nishimura, 1991; Moffet & Bridger, 1970). An ordered bi-bi mechanism, Equation (3), was used to model the reverse reaction with substrate A as succinate and substrate B as CoA. The parameters shown in Table IX were used for Reaction 9 of the model.

Table IX. Kinetic parameters utilized for Reaction 9 in the model.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Units</th>
<th>Organism</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fwd Rxn k</td>
<td>1</td>
<td>s⁻</td>
<td>NA</td>
<td>NA</td>
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<td>K_A</td>
<td>590</td>
<td>µM</td>
<td>E. coli</td>
<td>Luo &amp; Nishimura, 1991</td>
</tr>
<tr>
<td></td>
<td>620</td>
<td>µM</td>
<td>E. coli</td>
<td>Luo &amp; Nishimura, 1991</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>µM</td>
<td>E. coli</td>
<td>Joyce et al., 1999</td>
</tr>
<tr>
<td>Rev Rxn K_B</td>
<td>7.6</td>
<td>µM</td>
<td>E. coli</td>
<td>Luo &amp; Nishimura, 1991</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>µM</td>
<td>E. coli</td>
<td>Luo &amp; Nishimura, 1991</td>
</tr>
<tr>
<td></td>
<td>4.0</td>
<td>µM</td>
<td>E. coli</td>
<td>Joyce et al., 1999</td>
</tr>
<tr>
<td>k_cat</td>
<td>24.52</td>
<td>s⁻</td>
<td>E. coli</td>
<td>Joyce et al., 1999</td>
</tr>
</tbody>
</table>

Reaction 10:

\[
\text{Succinate} + \text{UQH}_2 \xrightarrow{\text{Succinate dehydrogenase}} \text{Fumarate} + \text{UQ} + 2\text{H}^+
\]

Enzyme: Succinate dehydrogenase

Succinate dehydrogenase catalyzes the oxidation of succinate to fumarate under aerobic conditions. It can also catalyze the reverse reaction, the reduction of fumarate to succinate. Succinate dehydrogenase requires flavins, ubiquinone, or menaquinol as electron donors and acceptors.

Forward Reaction:

The oxidation of succinate to fumarate has demonstrated normal Michaelis-Menten kinetics in studies of \textit{E. coli} (Cecchini \textit{et al}, 2002; Maklashina \textit{et al}, 2006). One
study indicated that succinate dehydrogenase was inhibited by oxaloacetate and malonate, but no mechanism was identified (Maklashina et al, 2006). This model utilized a noncompetitive inhibition mechanism, Equation (7), with oxaloacetate as the inhibitor.

Kinetic parameters utilized in this model are shown in Table X.

$$

v = \frac{V_{\text{MAX}}[S]}{(K_M + [S])^*\left(1 + \frac{I}{K_i}\right)} = \frac{k_{\text{cat}}*[E]*[S]}{(K_M + [S])^*\left(1 + \frac{I}{K_i}\right)}

$$

\begin{align*}
V_{\text{MAX}} &= k_{\text{cat}}*[E] = [\mu M/s] \\
K_M &= \text{Substrate concentration resulting in } 0.5V_{\text{MAX}} = [\mu M] \\
K_i &= \text{Inhibitor concentration in } 0.5V_{\text{MAX}} = [\mu M] \\
k_{\text{cat}} &= \text{Maximum number of rxns catalyzed per second } = [s^{-1}] \\
[S] &= \text{Concentration of substrate } = [\mu M] \\
[E] &= \text{Concentration of enzyme } = [\mu M] \\
[I] &= \text{Concentration of inhibitor } = [\mu M]
\end{align*}

**Reverse Reaction:**

The reduction of fumarate to succinate has displayed normal Michaelis-Menten kinetics in _E. coli_ (Cecchini et al, 2002; Maklashina et al, 2006). This model utilized Equation (4) to simulate this reaction. Kinetic parameters utilized in this model are shown in Table X.

**Table X. Kinetic parameters utilized for Reaction 10 in the model.**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Units</th>
<th>Organism</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Fwd Rxn</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$K_m$</td>
<td>110</td>
<td>$\mu M$</td>
<td><em>E. coli</em></td>
<td>Maklashina et al, 2006</td>
</tr>
<tr>
<td>$K_i$</td>
<td>0.07</td>
<td>$\mu M$</td>
<td><em>E. coli</em></td>
<td>Maklashina et al, 2006</td>
</tr>
<tr>
<td>$k_{\text{cat}}$</td>
<td>110</td>
<td>$s^{-1}$</td>
<td><em>E. coli</em></td>
<td>Maklashina et al, 2006</td>
</tr>
<tr>
<td></td>
<td>85</td>
<td>$s^{-1}$</td>
<td><em>E. coli</em></td>
<td>Cecchini et al, 2002</td>
</tr>
<tr>
<td><em>Rev Rxn</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$K_m$</td>
<td>100</td>
<td>$\mu M$</td>
<td><em>E. coli</em></td>
<td>Maklashina et al, 2006</td>
</tr>
<tr>
<td>$k_{\text{cat}}$</td>
<td>2</td>
<td>$s^{-1}$</td>
<td><em>E. coli</em></td>
<td>Maklashina et al, 2006</td>
</tr>
<tr>
<td></td>
<td>1.7</td>
<td>$s^{-1}$</td>
<td><em>E. coli</em></td>
<td>Cecchini et al, 2002</td>
</tr>
</tbody>
</table>
**Reaction 11:**

\[
\text{Fumarate} + \text{H}_2\text{O} \xleftrightarrow{\text{Fumarase}} \text{Malate}
\]

*Enzyme: Fumarase*

Fumarase catalyzes the reaction between fumarate and malate in the TCA cycle. There are three fumarase isozymes in *E. coli*. Fumarase A has the most activity under microaerophilic condition and is inactivated under aerobic conditions. Fumarase B has some activity in microaerophilic and aerobic conditions. Fumarase B also has a higher affinity for malate than fumarate. Fumarase C is highly active under aerobic conditions (Woods *et al*, 1988).

*Forward and Reverse Reaction:*

No kinetic mechanism was identified for Reaction 11. Normal Michaelis-Menten kinetics were assumed for the forward and reverse reactions. Equation (4) and the kinetic parameters in Table XI were utilized in the model of the forward and reverse reactions.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Units</th>
<th>Organism</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fwd Rxn</td>
<td>(K_m)</td>
<td>100 µM</td>
<td><em>E. coli</em></td>
<td>Rose &amp; Weaver, 2004</td>
</tr>
<tr>
<td></td>
<td>(k_{cat})</td>
<td>60 s⁻</td>
<td><em>E. coli</em></td>
<td>Rose &amp; Weaver, 2004</td>
</tr>
<tr>
<td>Rev Rxn</td>
<td>(K_m)</td>
<td>300 µM</td>
<td><em>E. coli</em></td>
<td>Rose &amp; Weaver, 2004</td>
</tr>
<tr>
<td></td>
<td>(k_{cat})</td>
<td>129 s⁻</td>
<td><em>E. coli</em></td>
<td>Rose &amp; Weaver, 2004</td>
</tr>
</tbody>
</table>

**Reaction 12:**

\[
\text{Malate} + \text{NAD}^+ \xleftrightarrow{\text{Malate dehydrogenase}} \text{Oxaloacetate} + \text{NADH} + \text{H}^+
\]
**Enzyme: Malate dehydrogenase**

Malate dehydrogenase catalyzes the reversible oxidation of malate to oxaloacetate. The enzyme uses NAD$^+$ as an electron acceptor. The activity of malate dehydrogenase is lower under anaerobic conditions.

**Forward Reaction:**

Studies indicate that this reaction follows an ordered bi-bi mechanism (Heyde & Ainsworth, 1968; Muslin *et al.*, 1995). Equation (3) was used to model the forward reaction with substrate A as malate and substrate B as NAD$^+$. The kinetic parameters found in Table XII were utilized for the forward reaction in the model.

**Reverse Reaction:**

It was assumed that the reverse reaction would also follow an ordered bi-bi mechanism, so Equation (3) was employed to model the reaction with substrate A as oxaloacetate and substrate B as NADH. The kinetic parameters found in Table XII were used to model this reaction.

<table>
<thead>
<tr>
<th>Table XII. Kinetic parameters utilized for Reaction 12 in the model.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Parameter</strong></td>
</tr>
<tr>
<td><strong>Fwd Rxn</strong></td>
</tr>
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<td></td>
</tr>
<tr>
<td><strong>Rev Rxn</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>
The glyoxylate pathway is a shunt in the TCA cycle. It is composed of two reactions catalyzed by the enzymes isocitrate lyase and malate synthase shown in Figure 3. Using additional acetyl-CoA, this pathway can convert isocitrate into malate and succinate bypassing the conversion into 2-oxoglutarate and succinyl-CoA. This shunt generates intermediates to be used elsewhere in the cell, but fails to generate the energy and reducing power that is generated when isocitrate continues through the TCA cycle. The glyoxylate shunt is included in this model to more accurately represent the energy and reducing power generated in the TCA cycle.

Figure 3. The metabolic pathway for the glyoxylate shunt in the TCA cycle.
**Reaction 13:**

\[ \text{Isocitrate lyase} \quad \text{Isocitrate} \leftrightarrow \text{Glyoxylate} + \text{Succinate} \]

**Enzyme: Isocitrate lyase**

Isocitrate lyase catalyzes the cleavage of isocitrate into glyoxylate and succinate. This enzyme diverts isocitrate from the TCA cycle into the glyoxylate shunt.

**Forward Reaction:**

Isocitrate lyase cleaves isocitrate into glyoxylate and succinate in the forward direction. The pH in *E. coli* cells is around 7.3 to 7.6 (Mackintosh & Nimmo, 1988); therefore, the parameters measured at pH = 7.3 were utilized in the model. Phosphoenolpyruvate acts as a non-competitive inhibitor, but the $K_i$ value suggests it is not significant *in vivo*. The species 3-phosphoglycerate is a competitive inhibitor of isocitrate lyase and is more significant due to its higher concentrations in the cell (Mackintosh & Nimmo, 1988). Equation (8) was used to model the competitive inhibition mechanism with 3-phosphoglycerate utilizing the parameters found in Table XIII.

\[
V = \frac{V_{\text{MAX}}[S]}{K_M \left(1 + \frac{I}{K_i}\right) + [S]} = \frac{k_{\text{cat}}[E][S]}{K_M \left(1 + \frac{I}{K_i}\right) + [S]}
\]

(8)

- $V_{\text{MAX}} = k_{\text{cat}}[E] = [\mu M/s]$
- $K_M = \text{Substrate concentration resulting in } 0.5V_{\text{MAX}} = [\mu M]$
- $K_i = \text{Inhibitor concentration in } 0.5V_{\text{MAX}} = [\mu M]$
- $k_{\text{cat}} = \text{Maximum number of rxns catalyzed per second} = [s^{-1}]$
- $[S] = \text{Concentration of substrate} = [\mu M]$
- $[E] = \text{Concentration of enzyme} = [\mu M]$
- $[I] = \text{Concentration of inhibitor} = [\mu M]$
Reverse Reaction:

Isocitrate lyase condenses glyoxylate and succinate into isocitrate in the reverse reaction. This reaction occurs by a sequential random-order equilibrium mechanism where the substrate binding of glyoxylate and succinate are independent (Mackintosh & Nimmo, 1988). Equation (5) and the parameters in Table XIII were used to model the reaction with substrate A as succinate and substrate B as glyoxylate.

Reaction 14:

\[
\text{Glyoxylate + Acetyl-CoA + H}_2\text{O} \xrightarrow{\text{Malate synthase}} \text{Malate + CoA + H}^+ 
\]

Enzyme: Malate synthase

Malate synthase exists as two isozymes in *E. coli*. Malate synthase A is the enzyme utilized in the glyoxylate shunt in the TCA cycle. Malate synthase A catalyzes
the irreversible reaction of glyoxylate and acetyl-CoA into malate. Malate synthase B catalyzes the synthesis of glyoxylate from glycolate (Molina et al., 1994).

**Forward Reaction:**

Malate synthase catalyzes the Claisen condensation of glyoxylate and acetyl-CoA into a malyl-CoA intermediate. The malyl-CoA intermediate is then cleaved into the two products, malate and CoA. Pyruvate is a competitive inhibitor to malate synthase (Anstrom et al., 2003). Studies indicate that this reaction proceeds through a random sequential mechanism with independent substrate binding. Equation (5) and the kinetic parameters shown Table XIV were used to model the reaction with substrate A as glyoxylate and substrate B as acetyl-CoA.

Table XIV. Kinetic parameters utilized for Reaction 14 in the model.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Units</th>
<th>Organism</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fwd Rxn</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$K_A$</td>
<td>21</td>
<td>µM</td>
<td><em>E. coli</em></td>
<td>Anstrom et al, 2003</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>µM</td>
<td><em>S. cerevisiae</em></td>
<td>Durchschlag et al, 1981</td>
</tr>
<tr>
<td>$K_B$</td>
<td>9.0</td>
<td>µM</td>
<td><em>E. coli</em></td>
<td>Anstrom et al, 2003</td>
</tr>
<tr>
<td></td>
<td>83</td>
<td>µM</td>
<td><em>S. cerevisiae</em></td>
<td>Durchschlag et al, 1981</td>
</tr>
<tr>
<td>$K_i$</td>
<td>1000</td>
<td>µM</td>
<td><em>E. coli</em></td>
<td>Anstrom et al, 2003</td>
</tr>
<tr>
<td>$\alpha$</td>
<td>1.0</td>
<td>-</td>
<td><em>S. cerevisiae</em></td>
<td>Durchschlag et al, 1981</td>
</tr>
<tr>
<td>$k_{cat}$</td>
<td>48.1</td>
<td>s$^{-1}$</td>
<td><em>E. coli</em></td>
<td>Anstrom et al, 2003</td>
</tr>
</tbody>
</table>
Glycolysis/Gluconeogenesis

Glycolysis is the metabolic pathway that breaks down glucose into pyruvate while generating a small amount of energy and reducing power. Glycolysis is composed of ten reactions seen in Figure 4. The enzymes that catalyze these ten reactions are glucokinase, phosphoglucone isomerase, 6-phosphofructokinase, fructose-bisphosphate aldolase, triose phosphate isomerase, glyceraldehyde-3-phosphate dehydrogenase, phosphoglycerate kinase, phosphoglycerate mutase, enolase, and pyruvate kinase. Cofactors that participate in glycolysis are ADP/ATP, NADH/NAD\(^+\), and P\(_i\). Glycolysis was included in this model because glucose is a common substrate used to grow cells and produce PHB.

Gluconeogenesis is a metabolic pathway that can convert pyruvate back into glucose. It includes many of the same enzymes as glycolysis. Gluconeogenesis uses five additional enzymes: glucose-1-phosphatase, phosphoglucomutase, fructose-1,6-bisphosphatase, phosphoenolpyruvate carboxykinase, and phosphoenolpyruvate synthetase. Gluconeogenesis includes 12 reactions as shown in Figure 4. Gluconeogenesis was included in this model to account for the conversion of pyruvate into glucose. Gluconeogenesis can occur when the concentrations of acetyl-CoA and citrate are high and the concentration of glucose is low.

**Reaction 15:**

*Forward Reaction:*

\[
\text{Glucose + ATP} \xrightarrow{\text{Glucokinase}} \text{Glucose-6-phosphate + ADP + P}_i
\]
The forward reaction is catalyzed by the enzyme glucokinase. Glucokinase phosphorylates glucose into the product glucose-6-phosphate. The reaction requires ATP to proceed. The kinetic mechanism for glucokinase has been studied in several organisms such as *Z. mobilis* and *P. shermanii*. It is hypothesized that the mechanism will be the same in *E. coli* because it is consistent with the crystal structure of the *E. coli* glucokinase. Studies indicate that the mechanism is a preferred order of substrate addition and product release. Glucose is added first followed by ATP (Lunin *et al.*, 2004). An ordered bi-bi mechanism, Equation (3), was used to model the forward reaction with the kinetics parameters found in Table XV.

*Reverse Reaction:*

The conversion of glucose-6-phosphate back into glucose is a two step process that involves two enzymes: phosphoglucomutase and glucose-1-phosphatase.

*Step 1:*

\[
\text{Glucose-6-phosphate} \xrightarrow{\text{Phosphoglucomutase}} \text{Glucose-1-phosphate}
\]

Step one is a reversible reaction catalyzed by phosphoglucomutase where glucose-6-phosphate is converted into glucose-1-phosphate via the intermediate glucose-1,6-diphosphate. No kinetic data were found for *E. coli* in the direction of glucose-6-phosphate to glucose-1-phosphate, but there was kinetic data from phosphoglucomutase in rat heart cells. Normal Michaelis-Menten kinetics, Equation (4), were assumed and kinetic parameters found in Table XV were utilized in the model.
A thermophilic phosphoglucomutase that was similar to the *E. coli* enzyme demonstrated a ping-pong catalytic mechanism in the direction of glucose-1-phosphate to glucose-6-phosphate (Yoshizaki *et al.*, 1971). Other studies have also suggested a ping-pong mechanism (Ray & Roscelli, 1964). It was decided to use normal Michaelis-Menten kinetics for the reverse direction of Step 1 to simplify the model. Equation (4) was utilized along with the kinetic parameters found in Table XV.

*Step 2:*

\[
\text{Glucose-1-phosphate} + \text{H}_2\text{O} \xrightarrow{\text{Glucose-1-phosphatase}} \text{Glucose} + \text{P}^i
\]

Step 2 is an irreversible reaction where glucose-1-phosphate is converted into glucose by the enzyme glucose-1-phosphatase. Normal Michaelis-Menten kinetics, Equation (4), were used to model the reaction with the parameters found in Table XV.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Units</th>
<th>Organism</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fwd Rxn</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$K_A$</td>
<td>780</td>
<td>µM</td>
<td><em>E. coli</em></td>
<td>Meyer <em>et al.</em>, 1997</td>
</tr>
<tr>
<td>$K_B$</td>
<td>3760</td>
<td>µM</td>
<td><em>E. coli</em></td>
<td>Meyer <em>et al.</em>, 1997</td>
</tr>
<tr>
<td>$V_{m}$</td>
<td>2.643</td>
<td>µM/s</td>
<td><em>E. coli</em></td>
<td>Meyer <em>et al.</em>, 1997</td>
</tr>
<tr>
<td><strong>Rev Rxn</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$K_M$</td>
<td>670</td>
<td>µM</td>
<td>Rat heart</td>
<td>Kashiwaya <em>et al.</em>, 1994</td>
</tr>
<tr>
<td>$V_{m}$</td>
<td>1.12</td>
<td>µM/s</td>
<td>Rat heart</td>
<td>Kashiwaya <em>et al.</em>, 1994</td>
</tr>
<tr>
<td><strong>Step 1 Fwd</strong></td>
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<td></td>
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</tr>
<tr>
<td>$K_m$</td>
<td>60</td>
<td>µM</td>
<td><em>E. coli</em></td>
<td>Josh &amp; Handler, 1964</td>
</tr>
<tr>
<td>$V_{m}$</td>
<td>6700</td>
<td>µM</td>
<td><em>E. coli</em></td>
<td>Dworniczak <em>et al.</em>, 2008</td>
</tr>
<tr>
<td><strong>Rev Rxn</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$K_m$</td>
<td>0.001</td>
<td>µM/s</td>
<td><em>E. coli</em></td>
<td>Dworniczak <em>et al.</em>, 2008</td>
</tr>
<tr>
<td><strong>Step 1 Rev</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$K_m$</td>
<td>240</td>
<td>µM</td>
<td><em>E. coli</em></td>
<td>Kuznetsova <em>et al.</em>, 2006</td>
</tr>
<tr>
<td>$k_{cat}$</td>
<td>1.4</td>
<td>s$^{-1}$</td>
<td><em>E. coli</em></td>
<td>Kuznetsova <em>et al.</em>, 2006</td>
</tr>
</tbody>
</table>

**Reaction 16:**

\[
\text{Glucose-6-phosphate} \xleftrightarrow{\text{Phosphoglucoisomerase}} \text{Fructose-6-phosphate}
\]
**Enzyme: Phosphoglucone isomerase**

Reaction 16 is a reversible reaction catalyzed by the enzyme phosphoglucone isomerase. The enzyme converts glucose-6-phosphate into fructose-6-phosphate or vice versa.

**Forward and Reverse Reaction:**

A study in *E. coli* indicated that the kinetic mechanism utilized in this reaction was a uni-uni reversible that utilized the Haldane relationship (Ishii *et al*., 2007). Equation (9) and the kinetic parameters shown in Table XVI were used to model this reaction.

Glucose-6-phosphate was used as substrate A, and fructose-6-phosphate was used as substrate B. The reverse reaction uses the same kinetics as the forward reaction (Ishii *et al*., 2007); therefore, it was modeled using the same equation and parameters.

\[
V = \frac{V_{\text{MAX}} \left( \frac{[A] - [B]}{K_{eq}} \right)}{K_A \left( 1 + \frac{[B]}{K_B} \right) + [A]} = \frac{k_{\text{cat}} * [E] * \left( \frac{[A] - [B]}{K_{eq}} \right)}{K_A \left( 1 + \frac{[B]}{K_B} \right) + [A]}
\]

\[
V_{\text{MAX}} = k_{\text{cat}} * [E] = [\mu\text{M/s}]
\]

\[
K_A = \text{Dissociation constant for substrate A} = [\mu\text{M}]
\]

\[
K_B = \text{Dissociation constant for substrate B} = [\mu\text{M}]
\]

\[
k_{\text{cat}} = \text{Maximum number of rxns catalyzed per second} = [\text{s}^{-1}]
\]

\[
K_{\text{eq}} = \text{Equilibrium constant [unitless]}
\]

\[
[A] = \text{Concentration of substrate A} = [\mu\text{M}]
\]

\[
[B] = \text{Concentration of substrate B} = [\mu\text{M}]
\]

\[
[E] = \text{Concentration of enzyme} = [\mu\text{M}]
\]

**Table XVI. Kinetic parameters utilized for Reaction 16 in the model.**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Units</th>
<th>Organism</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fwd</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$K_A$</td>
<td>3000</td>
<td>$\mu$M</td>
<td><em>E. coli</em> K-12</td>
<td>Ishii <em>et al</em>, 2007</td>
</tr>
<tr>
<td>$K_B$</td>
<td>160</td>
<td>$\mu$M</td>
<td><em>E. coli</em> K-12</td>
<td>Ishii <em>et al</em>, 2007</td>
</tr>
<tr>
<td><strong>Rxn</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$K_{eq}$</td>
<td>0.3</td>
<td></td>
<td><em>E. coli</em> K-12</td>
<td>Ishii <em>et al</em>, 2007</td>
</tr>
<tr>
<td>$V_m$</td>
<td>25.18</td>
<td>$\mu$M/s</td>
<td><em>E. coli</em> K-12</td>
<td>Ishii <em>et al</em>, 2007</td>
</tr>
</tbody>
</table>
**Reaction 17:**

*Forward Reaction:*

\[
\text{Fructose-6-phosphate} + \text{ATP} \xrightarrow{6\text{-phosphofructokinase}} \text{Fructose-1,6-bisphosphate} + \text{ADP} + 2\text{H}^+ 
\]

*Enzyme: 6-phosphofructokinase*

The ATP dependent 6-phosphofructokinase catalyzes the phosphorylation of fructose-6-phosphate into fructose-1,6-bisphosphate. An ordered bi-bi reaction mechanism has been proposed for this reaction in *E. coli* with fructose-6-phosphate as substrate A and ATP as substrate B (Campos *et al.*, 1984). Equation (3) and the kinetic parameters shown in Table XVII were utilized in the model of the forward direction of Reaction 17.

*Reverse Reaction:*

\[
\text{Fructose-1,6-bisphosphate} + \text{H}_2\text{O} \xrightarrow{\text{Fructose-1,6-bisphosphatase}} \text{Fructose-6-phosphate} + \text{P}^i 
\]

*Enzyme: Fructose-1,6-bisphosphatase*

Fructose-1,6-bisphosphatase catalyzes the dephosphorylation of fructose-1,6-bisphosphate to fructose-6-phosphate. There are four major genes that encode for fructose-1,6-bisphosphatase in *E. coli*: *fbp*, *yphA*, *yggF*, *glpX*. Fbp is the main fructose-1,6-bisphosphatase in the cell. YggF and GlpX are type II fructose-1,6-bisphosphatases.

Hill kinetics have been shown for this reaction in *E. coli* with a cooperativity factor of 2.0 (Brown *et al.*, 2009). Equation (6) and the kinetic parameters shown in Table XVII were used to model the reverse direction of Reaction 17. Values for all four
fructose-1,6-bisphosphatases are shown in Table XVII but only values for Fbp were used in the model.

### Table XVII. Kinetic parameters utilized for Reaction 17 in the model.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Units</th>
<th>Organism</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>( K_A )</td>
<td>107</td>
<td>( \mu M )</td>
<td><em>E. coli</em></td>
<td>Wang &amp; Kemp, 1999</td>
</tr>
<tr>
<td>( K_B )</td>
<td>32</td>
<td>( \mu M )</td>
<td><em>E. coli</em></td>
<td>Campos <em>et al</em>, 1984</td>
</tr>
<tr>
<td>( k_{\text{cat}} )</td>
<td>210</td>
<td>( \mu M )</td>
<td><em>E. coli</em></td>
<td>Wang &amp; Kemp, 1999</td>
</tr>
<tr>
<td>( \text{Fwd Rxn} )</td>
<td>20</td>
<td>( \mu M )</td>
<td><em>E. coli</em></td>
<td>Campos <em>et al</em>, 1984</td>
</tr>
<tr>
<td>( K_m )</td>
<td>20(^a)</td>
<td>( \mu M )</td>
<td><em>E. coli</em></td>
<td>Brown <em>et al</em>, 2009</td>
</tr>
<tr>
<td>( V_m )</td>
<td>0.403(^a)</td>
<td>( \mu M /s )</td>
<td><em>E. coli</em></td>
<td>Brown <em>et al</em>, 2009</td>
</tr>
<tr>
<td>( k_{\text{cat}} )</td>
<td>14.6(^a)</td>
<td>( s^{-1} )</td>
<td><em>E. coli</em></td>
<td>Brown <em>et al</em>, 2009</td>
</tr>
<tr>
<td>( n )</td>
<td>2.0</td>
<td>-</td>
<td><em>E. coli</em></td>
<td>Brown <em>et al</em>, 2009</td>
</tr>
</tbody>
</table>

\(^a\) Fbp fructose-1,6-bisphosphatase  
\(^b\) YbhA fructose-1,6-bisphosphatase  
\(^c\) YggF fructose-1,6-bisphosphatase  
\(^d\) GlpX fructose-1,6-bisphosphatase

### Reaction 18:

\[
\text{Fructose-1,6-bisphosphate} \quad \leftrightarrow \quad \text{Fructose-bisphosphate aldolase} \\
\text{Fructose-1,6-bisphosphate aldolase} \quad \rightarrow \quad \text{Dihydroxyacetone phosphate} + \text{Glyceraldehyde-3-phosphate}
\]

*Enzyme: Fructose-bisphosphate aldolase*

Fructose-bisphosphate aldolase catalyzes the aldol cleavage of fructose-1,6-bisphosphate into dihydroxyacetone phosphate and glyceraldehyde-3-phosphate.
**Forward Reaction:**

Normal Michaelis-Menten kinetics were observed for the cleavage of fructose-1,6-bisphosphate in *E. coli* (Plater et al., 1999). Equation (4) and the kinetic parameters shown in Table XVIII were used to model this reaction.

**Reverse Reaction:**

The reverse reaction proceeds via a ping-pong mechanism (Lambeth & Kushmerick, 2002). Equation (2) and the kinetic parameters in Table XVIII were used to model the reverse reaction in SimBiology. Substrate A was dihydroxyacetone phosphate and substrate B was glyceraldehyde-3-phosphate in the model.

Table XVIII. Kinetic parameters utilized for Reaction 18 in the model.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Units</th>
<th>Organism</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fwd K&lt;sub&gt;m&lt;/sub&gt;</td>
<td>230</td>
<td>µM</td>
<td><em>E. coli</em></td>
<td>Plater et al., 1999</td>
</tr>
<tr>
<td>Fwd k&lt;sub&gt;cat&lt;/sub&gt;</td>
<td>0.07</td>
<td>s&lt;sup&gt;-1&lt;/sup&gt;</td>
<td><em>E. coli</em></td>
<td>Plater et al., 1999</td>
</tr>
<tr>
<td>Rev K&lt;sub&gt;A&lt;/sub&gt;</td>
<td>2100</td>
<td>µM</td>
<td>Rabbit</td>
<td>Lambeth &amp; Kushmerick, 2002</td>
</tr>
<tr>
<td>Rev K&lt;sub&gt;B&lt;/sub&gt;</td>
<td>1100</td>
<td>µM</td>
<td>Rabbit</td>
<td>Lambeth &amp; Kushmerick, 2002</td>
</tr>
<tr>
<td>Rev V&lt;sub&gt;m&lt;/sub&gt;</td>
<td>1733</td>
<td>µM /s</td>
<td>Rabbit</td>
<td>Lambeth &amp; Kushmerick, 2002</td>
</tr>
</tbody>
</table>

**Reaction 19:**

Dihydroxyacetone phosphate $\xrightarrow{\text{Triose phosphate isomerase}}$ Glyceraldehyde-3-phosphate

**Enzyme: Triose phosphate isomerase**

Triose phosphate isomerase catalyzes the conversion of dihydroxyacetone phosphate into glyceraldehyde-3-phosphate via a cis-ene-diolate intermediate. Triose phosphate isomerase is considered to be a perfect enzyme.
**Forward and Reverse Reaction:**

The forward and reverse reactions are expressed by normal Michaelis-Menten kinetics (Nickbarg & Knowles, 1988). Equation (4) and the parameters in Table XIX were used to model the reaction.

Table XIX. Kinetic parameters utilized for Reaction 19 in the model.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Units</th>
<th>Organism</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fwd Km</td>
<td>2300</td>
<td>µM</td>
<td>E. coli</td>
<td>Nickbarg &amp; Knowles, 1988</td>
</tr>
<tr>
<td>Fwd Keq</td>
<td>750</td>
<td>µM</td>
<td>E. coli</td>
<td>Nickbarg &amp; Knowles, 1988</td>
</tr>
<tr>
<td>Fwd kcat</td>
<td>750</td>
<td>s^-</td>
<td>E. coli</td>
<td>Nickbarg &amp; Knowles, 1988</td>
</tr>
<tr>
<td>Rev Km</td>
<td>320</td>
<td>µM</td>
<td>Rabbit</td>
<td>Lambeth &amp; Kushmerick, 2002</td>
</tr>
<tr>
<td>Rev Vm</td>
<td>200</td>
<td>µM /s</td>
<td>Rabbit</td>
<td>Lambeth &amp; Kushmerick, 2002</td>
</tr>
</tbody>
</table>

**Reaction 20:**

\[
\text{Glyceraldehyde-3-phosphate + P}^+ + \text{NAD}^+ \xrightarrow{\text{Glyceraldehyde-3-phosphate dehydrogenase}} 1,3\text{-diphosphoglycerate + NADH + H}^+
\]

**Enzyme: Glyceraldehyde-3-phosphate dehydrogenase**

The enzyme glyceraldehyde-3-phosphate dehydrogenase catalyzes the reversible oxidation of glyceraldehyde-3-phosphate into 1,3-diphosphoglycerate. The cofactor NAD$^+$ is reduced to NADH during the reaction.

**Forward Reaction:**

The forward reaction proceeds via a sequential ordered ter-bi mechanism (Wang & Alapovic, 1980). Ordered ter-bi reactions can be represented by the ordered ter-ter kinetic expression for steady-state and rapid equilibrium kinetics (Purich & Allison, 2000). Equation (10) and the kinetic parameters found in Table XX were used to model
the forward reaction in SimBiology. In the equation, substrate A was NAD$^+$, substrate B was glyceraldehyde-3-phosphate, and substrate C was inorganic phosphate.

$$v = \frac{V_{\text{MAX}}}{K_A + \frac{\left( K_B K_C \right)}{[A][B][C]} + \frac{\left( K_A K_B \right)}{[A][B]} + \frac{K_B K_C}{[B][C]} + \frac{K_A K_B}{[A][B]} + \frac{K_B}{[B]} + \frac{K_A}{[A]} + 1}$$

$V_{\text{MAX}} = k_{\text{cat}}\times [E] = [\mu\text{M/s}]$
$K_A = $ Dissociation constant for substrate A = [µM]
$K_B = $ Dissociation constant for substrate B = [µM]
$K_C = $ Dissociation constant for substrate C = [µM]
$K_{iA} = $ Binding constant for substrate A = [µM]
$K_{iB} = $ Binding constant for substrate B = [µM]
$K_{iC} = $ Binding constant for substrate C = [µM]
$k_{\text{cat}} = $ Maximum number of rxns catalyzed per second = [s$^{-1}$]
$[A] = $ Concentration of substrate A = [µM]
$[B] = $ Concentration of substrate B = [µM]
$[C] = $ Concentration of substrate C = [µM]
$[E] = $ Concentration of enzyme = [µM]

### Table XX. Kinetic parameters utilized for Reaction 20 in the model.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Units</th>
<th>Organism</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_A$</td>
<td>90</td>
<td>µM</td>
<td>Rabbit</td>
<td>Lambeth &amp; Kushmerick, 2002</td>
</tr>
<tr>
<td>$K_B$</td>
<td>290</td>
<td>µM</td>
<td>S. mutans</td>
<td>Crow &amp; Wittenberger, 1979</td>
</tr>
<tr>
<td>$K_C$</td>
<td>290</td>
<td>µM</td>
<td>Rabbit</td>
<td>Lambeth &amp; Kushmerick, 2002</td>
</tr>
<tr>
<td>$V_m$</td>
<td>0.38</td>
<td>µM/s</td>
<td>S. mutans</td>
<td>Crow &amp; Wittenberger, 1979</td>
</tr>
</tbody>
</table>

**Reverse Reaction:**

The reverse reaction has a sequential ordered bi-ter mechanism (Wang & Alaupovic, 1980). Ordered bi-ter reactions can be represented by the ordered bi-bi kinetic expression for steady-state and rapid equilibrium kinetics (Purich & Allison, 2000).

Equation (3) and the kinetic parameters found in Table XX were used to model the
forward reaction in SimBiology. Substrate A was set as 1,3-diphosphoglycerate and substrate B was set as NADH.

**Reaction 21:**

\[ \text{1,3-diphosphoglycerate} + \text{ADP} + H^+ \xleftrightarrow{\text{Phosphoglycerate kinase}} \text{3-phosphoglycerate} + \text{ATP} \]

*Enzyme: Phosphoglycerate kinase*

Phosphoglycerate kinase catalyzes the phosphoryl group transfer from 1,3-diphosphoglycerate to ADP to form ATP and 3-phosphoglycerate.

*Forward Reaction:*

The forward reaction catalyzed by phosphoglycerate kinase follows a sequential random bi-bi mechanism (Lavoinne *et al.*, 1983). Equation (5) and the kinetic parameters in Table XXI were used to model the reaction. Substrate binding was assumed to be independent with substrate A as 1,3-diphosphoglycerate and substrate B as ADP.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Units</th>
<th>Organism</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fwd Rxn</td>
<td>$K_A$</td>
<td>2.2 µM</td>
<td>Rabbit</td>
<td>Lambeth &amp; Kushmerick, 2002</td>
</tr>
<tr>
<td>Fwd Rxn</td>
<td>$K_B$</td>
<td>50 µM</td>
<td>Pig</td>
<td>Lambeth &amp; Kushmerick, 2002</td>
</tr>
<tr>
<td>Fwd Rxn</td>
<td>$V_m$</td>
<td>18.7 µM/s</td>
<td>Pig</td>
<td>Lambeth &amp; Kushmerick, 2002</td>
</tr>
<tr>
<td>Rev Rxn</td>
<td>$K_A$</td>
<td>1200 µM</td>
<td>Pig</td>
<td>Lambeth &amp; Kushmerick, 2002</td>
</tr>
<tr>
<td>Rev Rxn</td>
<td>$K_B$</td>
<td>360 µM</td>
<td>Rabbit</td>
<td>Lambeth &amp; Kushmerick, 2002</td>
</tr>
<tr>
<td>Rev Rxn</td>
<td>$V_m$</td>
<td>18.7 µM/s</td>
<td>Pig</td>
<td>Lambeth &amp; Kushmerick, 2002</td>
</tr>
</tbody>
</table>

*Reverse Reaction:*

The reverse reaction catalyzed by phosphoglycerate kinase also follows a sequential random bi-bi mechanism (Lavoinne *et al.*, 1983). Equation (5) and the kinetic parameters in Table XXI were used to model the reaction. Substrate binding was assumed to be independent with substrate A as 1,3-diphosphoglycerate and substrate B as ADP.
parameters in Table XXI were used to model the reaction. Substrate binding was assumed to be independent with substrate A as 3-phosphoglycerate and substrate B as ATP.

**Reaction 22:**

\[
3\text{-phosphoglycerate} + \text{H}^+ \overset{\text{Phosphoglycerate mutase}}{\rightleftharpoons} 2\text{-phosphoglycerate}
\]

*Enzyme: Phosphoglycerate mutase*

Phosphoglycerate mutase catalyzes the intramolecular phosphoryl group transfer to form 2-phosphoglycerate from 3-phosphoglycerate. A histidine-phosphoenzyme intermediate is formed during the reaction.

*Forward and Reverse Reaction:*

No kinetic mechanism was identified in the literature for Reaction 22; therefore, normal Michaelis-Menten kinetics were assumed for the reaction in SimBiology.

Equation (4) and the kinetic parameters in Table XXII were used to model Reaction 22.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Units</th>
<th>Organism</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fwd Km</td>
<td>200</td>
<td>µM</td>
<td><em>E. coli dPGM</em></td>
<td>Fraser <em>et al.</em>, 1999</td>
</tr>
<tr>
<td>Rxn kcat</td>
<td>330</td>
<td>s⁻</td>
<td><em>E. coli dPGM</em></td>
<td>Fraser <em>et al.</em>, 1999</td>
</tr>
<tr>
<td></td>
<td>22</td>
<td>s⁻</td>
<td><em>E. coli iPGM</em></td>
<td>Fraser <em>et al.</em>, 1999</td>
</tr>
<tr>
<td>Rev Km</td>
<td>190</td>
<td>µM</td>
<td><em>E. coli dPGM</em></td>
<td>Fraser <em>et al.</em>, 1999</td>
</tr>
<tr>
<td>Rxn kcat</td>
<td>97</td>
<td>µM</td>
<td><em>E. coli iPGM</em></td>
<td>Fraser <em>et al.</em>, 1999</td>
</tr>
<tr>
<td></td>
<td>220</td>
<td>s⁻</td>
<td><em>E. coli dPGM</em></td>
<td>Fraser <em>et al.</em>, 1999</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>s⁻</td>
<td><em>E. coli iPGM</em></td>
<td>Fraser <em>et al.</em>, 1999</td>
</tr>
</tbody>
</table>
Reaction 23:

\[
\text{Enolase} \quad 2\text{-phosphoglycerate} \rightleftharpoons \text{Enolase} \quad \text{Phosphoenolpyruvate} + \text{H}_{2}\text{O} + \text{H}^{+}
\]

**Enzyme: Enolase**

Enolase catalyzes the dehydration of 2-phosphoglycerate that produces the high energy compound phosphoenolpyruvate.

**Forward and Reverse Reaction:**

No kinetic mechanism was identified for Reaction 23. Both reactions were assumed to proceed via normal Michaelis-Menten kinetics. Equation (4) and the kinetic parameters shown in Table XXIII were used to model the reaction in SimBiology.

Table XXIII. Kinetic parameters utilized for Reaction 23 in the model.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Units</th>
<th>Organism</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fwd K\text{m}</td>
<td>100</td>
<td>µM</td>
<td><em>E. coli</em></td>
<td>Spring &amp; Wold, 1971</td>
</tr>
<tr>
<td>Fwd V\text{m}</td>
<td>120</td>
<td>µM</td>
<td>Rat</td>
<td>Lambeth &amp; Kushmerick, 2002</td>
</tr>
<tr>
<td>Rev K\text{m}</td>
<td>370</td>
<td>µM</td>
<td>Rat</td>
<td>Lambeth &amp; Kushmerick, 2002</td>
</tr>
<tr>
<td>Rev V\text{m}</td>
<td>3.2</td>
<td>µM/s</td>
<td>Rabbit</td>
<td>Lambeth &amp; Kushmerick, 2002</td>
</tr>
</tbody>
</table>

Reaction 24:

**Forward Reaction:**

\[
\text{Phosphoenolpyruvate} + \text{ADP} + 2\text{H}^{+} \underset{\text{Pyruvate kinase}}{\rightarrow} \text{Pyruvate} + \text{ATP}
\]

**Enzyme: Pyruvate kinase**

The forward reaction is catalyzed by pyruvate kinase and has been shown to have a sequential random bi-bi mechanism (Giles *et al*, 1976; Waygood *et al*, 1976). Equation
and the kinetic parameters found in Table XXIV were used to model the reaction in SimBiology. Substrate A was set as phosphoenolpyruvate and substrate B was set as ADP.

**Reverse Reaction:**

\[
\text{Pyruvate} + \text{ATP} + \text{H}_2\text{O} \xrightarrow{\text{Phosphoenolpyruvate synthetase}} \text{Phosphoenolpyruvate} + \text{AMP} + 3\text{H}^+ + \text{P}^i
\]

*Enzyme: Phosphoenolpyruvate synthetase*

The reverse reaction is catalyzed by phosphoenolpyruvate synthetase and proceeds via a ping-pong mechanism (Sigman, 1990). Equation (2) and the values in Table XXIV were used in the model with substrate A as pyruvate and substrate B as ATP.

**Table XXIV. Kinetic parameters utilized for Reaction 24 in the model.**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Units</th>
<th>Organism</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_A$</td>
<td>25</td>
<td>$\mu$M</td>
<td><em>E. coli</em></td>
<td>Valentini <em>et al.</em>, 2000</td>
</tr>
<tr>
<td></td>
<td>80</td>
<td>$\mu$M</td>
<td>Rat</td>
<td>Lambeth &amp; Kushmerick, 2002</td>
</tr>
<tr>
<td>$K_B$</td>
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<td>$\mu$M</td>
<td><em>E. coli</em></td>
<td>Valentini <em>et al.</em>, 2000</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>$\mu$M</td>
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<td>Lambeth &amp; Kushmerick, 2002</td>
</tr>
<tr>
<td>$k_{cat}$</td>
<td>103 $s^{-1}$</td>
<td><em>E. coli</em></td>
<td>Valentini <em>et al.</em>, 2000</td>
<td></td>
</tr>
<tr>
<td></td>
<td>110 $s^{-1}$</td>
<td><em>E. coli</em></td>
<td>Valentini <em>et al.</em>, 2000</td>
<td></td>
</tr>
</tbody>
</table>

<table>
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<td>Rabbit</td>
<td>Lambeth &amp; Kushmerick, 2002</td>
<td></td>
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<tr>
<td>$K_B$</td>
<td>820 $\mu$M</td>
<td>Rabbit</td>
<td>Lambeth &amp; Kushmerick, 2002</td>
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</tr>
<tr>
<td>$V_m$</td>
<td>24 $\mu$M/s</td>
<td>Rabbit</td>
<td>Lambeth &amp; Kushmerick, 2002</td>
<td></td>
</tr>
</tbody>
</table>

**Reaction 25:**

\[
\text{Oxaloacetate} + \text{ATP} \xrightarrow{\text{Phosphoenolpyruvate carboxykinase}} \text{Phosphoenolpyruvate} + \text{CO}_2 + \text{ADP} + \text{H}^+
\]
Enzyme: Phosphoenolpyruvate carboxykinase

Phosphoenolpyruvate carboxykinase catalyzes the irreversible conversion of oxaloacetate into phosphoenolpyruvate via a sequential random bi-bi mechanism (Krebs & Bridger, 1980). Equation (5) and the kinetic parameters shown in Table XXV were used to model this reaction in SimBiology. Substrate binding was assumed to be independent with substrate A as oxaloacetate and substrate B as ATP.

Table XXV. Kinetic parameters utilized for Reaction 25 in the model.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Units</th>
<th>Organism</th>
<th>Reference</th>
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<tr>
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<td>µM</td>
<td>E. coli</td>
<td>Krebs &amp; Bridger, 1980</td>
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<tr>
<td>K_B</td>
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<td>µM</td>
<td>E. coli</td>
<td>Krebs &amp; Bridger, 1980</td>
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<td>V_m</td>
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<td>µM/s</td>
<td>E. coli</td>
<td>Krebs &amp; Bridger, 1980</td>
</tr>
</tbody>
</table>

Acetyl-CoA Synthesis

Acetyl-CoA synthesis is a metabolic pathway composed of one reaction. This reaction is catalyzed by the enzyme complex pyruvate dehydrogenase as shown in Figure 5. This reaction converts pyruvate into acetyl-CoA with the help of the cofactor CoA. This pathway was included in the model because of the use of acetyl-CoA as the starting substrate in the PHB synthesis pathway.

Figure 5. The metabolic pathway for acetyl-CoA synthesis.
Reaction 26:

\[
\text{Pyruvate} + \text{CoA} + \text{NAD}^+ \xrightarrow{\text{Pyruvate dehydrogenase}} \text{Acetyl-CoA} + \text{CO}_2 + \text{NADH}
\]

**Enzyme: Pyruvate dehydrogenase complex**

The pyruvate dehydrogenase complex catalyzes an irreversible reaction to produce acetyl-CoA from pyruvate, the product of glycolysis. It is a large, multienzyme complex composed of three distinct types of enzyme. The pyruvate dehydrogenase complex has several catalytic cofactors that include CoA, NAD\(^+\), thiamine pyrophosphate (TPP), lipoamide, and FAD. The complex reaction can be broken down into four steps.

**Step 1:**

In the first step, pyruvate is decarboxylated after it combines with TPP. This stage of the reaction is catalyzed by the pyruvate dehydrogenase component of the complex (E1). This step exhibits tight-binding inhibition with thiamine 2-thiazolone diphosphate (ThTDP), and with thiamine 2-thiothiazolone diphosphate (ThTTDP) (Liu & Bisswanger, 2003; Nemeria *et al.*, 2001).

\[
\text{Pyruvate} + \text{TPP} \xrightarrow{\text{E1}} \text{Hydroxyethyl-TPP} + \text{CO}_2
\]

**Step 2:**

In the second step, the hydroxyethyl attached to the TPP is oxidized to form an acetyl group that is transferred to lipoamide. This stage of the reaction is also catalyzed by the pyruvate dehydrogenase component of the complex (E1) (Liu & Bisswanger, 2003; Nemeria *et al.*, 2001).
Hydroxyethyl-TPP + Lipoamide $\xrightarrow{E_1}$ Carbanion of TPP + Acetyllipoamide

**Step 3:**

In the third step, the acetyl group is transferred from acetyllipoamide to CoA to form acetyl-CoA. Dihydrolipoyl transacetylase (E2) catalyzes this step (Snoep et al, 1992; Willms et al, 1967).

Acetyllipoamide + HS-CoA $\xrightarrow{E_2}$ Dihydrolipoamide + Acetyl-CoA

**Step 4:**

In the fourth step, the oxidized lipoamide is regenerated using NAD$^+$. This stage of the reaction is catalyzed by dihydrolipoyl dehydrogenase (E3) (Allison et al, 1988).

Dihydrolipoamide + NAD$^+$ $\xrightarrow{E_3}$ Lipoamide + NADH + H$^+$

**Overall Reaction:**

Kinetic studies on the pyruvate dehydrogenase complex from the bovine kidney have demonstrated that the overall reaction fits a random bi-bi mechanism as well as the Theorell-Chance mechanism (Butterworth et al, 1975).

In *E. coli*, studies have shown that the first step catalyzed by the E1 component of the enzyme complex is the rate-limiting step (Liu & Bisswanger, 2003). Michaelis-Menten kinetic parameters have been determined for the overall reaction in *E. coli*. Equation (4) and the parameters in Table XXVI were used to model the overall reaction in SimBiology.
Table XXVI. Kinetic parameters utilized for Reaction 26 in the model.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Units</th>
<th>Organism</th>
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</tr>
</thead>
<tbody>
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<td>321$^a$</td>
<td>$\mu$M</td>
<td>$E. coli$</td>
<td>Liu &amp; Bisswanger, 2003</td>
</tr>
<tr>
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<td>6.263$^a$</td>
<td>$\mu$M/s</td>
<td>$E. coli$</td>
<td>Liu &amp; Bisswanger, 2003</td>
</tr>
<tr>
<td>Rxn $V_m$</td>
<td>3.682$^b$</td>
<td>$\mu$M/s</td>
<td>$E. coli$</td>
<td>Liu &amp; Bisswanger, 2003</td>
</tr>
</tbody>
</table>

$a$. $\text{H}_2\text{O}$ as solvent

$b$. $\text{D}_2\text{O}$ as solvent
The transcriptional network associated with the enzymes in the selected metabolic pathways was included in the model. Enzymes are synthesized through the transcription and translation of genes. Cells regulate the amount of enzymes synthesized depending upon the needs of the cell. It is undesirable to waste energy synthesizing enzymes that are not needed. Transcription factors are regulatory proteins that can repress or induce transcription of genes. Cells use transcription factors to dynamically regulate the intracellular concentration of enzymes (Lehninger et al, 2008).

The first step in modeling the transcriptional network was to identify all the genes that contribute to the synthesis of the 30 enzymes used in Reactions 1-26. After the genes were known, then the next step was to determine which promoters initiated transcription of each gene. Next, the transcription factors associated with each promoter were identified. Only promoters that interact with transcription factors were included in the model. An equation was developed to calculate the rate of enzyme synthesis based on promoter activity and the concentrations of each transcription factor. Each promoter was represented by a reaction in the SimBiology model.

Ligand binding was also included in the model. Ligands that bind to each transcription factor were identified. Rules were used in SimBiology to calculate the concentration of active transcription factors based on the concentration of ligands. Reactions were also included to account for the degradation of enzymes that occurs in the cell. The end result of these equations and rules was a dynamic model of the concentration of 30 enzymes based on a transcriptional network.
Enzyme Genes and Promoters

The enzyme genes were determined using the EcoCyc database (ecocyc.org) and the RegulonDB database (regulondb.ccg.unam.mx) for *E. coli* K12. These databases were used to identify the promoters that initiate transcription of each gene, and the regulatory proteins that activate or repress the transcription of each gene.

**β-ketothiolase:**

The gene *phaA* encodes for the enzyme β-ketothiolase that catalyzes Reaction 1. The gene *phaA* is not naturally found in *E. coli*. The plasmid pBHR68 shown in Figure 6 has been used to insert *phaA*, *phaB*, and *phaC* into *E. coli* (Linton, 2010). These three genes are transcribed using the *lac* promoter shown in Figure 6. The transcription factors Crp, H-NS, and LacI help regulate *lac* as shown in Figure 7.

![Figure 6. Summary diagram of the pBHR68 plasmid (Spiekermann et al, 1999).](image)

![Figure 7. Transcription regulation summary diagram for the *lac* promoter from EcoCyc.](image)
**Acetoacetyl-CoA Reductase:**

The gene *phaB* encodes for the enzyme acetoacetyl-CoA reductase that catalyzes Reaction 2. The gene *phaB* is not found in the *E. coli* genome and is inserted using the plasmid pBHR68 shown in Figure 6. The promoter associated with *phaB* is the *lac* promoter. Figure 7 shows how Crp, H-NS, and LacI regulate the transcription of *phaB*.

**Poly-β-hydroxybutyrate Polymerase:**

The gene *phaC* encodes for the enzyme poly-β-hydroxybutyrate polymerase that catalyzes Reaction 3. The gene *phaC* is added into *E. coli* by using the plasmid pBHR68 shown in Figure 6. The *lac* promoter and the three transcription factors shown in Figure 7 (Crp, H-NS, and LacI) regulate the transcription of *phaC*.

**Citrate Synthase:**

Citrate synthase is the enzyme that catalyzes Reaction 4, and it is encoded in the *E. coli* genome by the gene *gltA*. The transcription factors that regulate the transcription of *gltA* are Crp, IHF, and ArcA as shown in Figure 8. Figure 9 illustrates that there are two promoters for *gltA*, but only the promoter *gltAp1* is affected by the transcription factors. Therefore, only the promoter *gltAp1* was included in the model.

![Figure 8](image-url)

**Figure 8.** Transcription regulation summary diagram for the gene *gltA* from EcoCyc.
Figure 9. Promoters and transcription factors for the gene gltA from EcoCyc.

**Citrate Hydro-lyase:**

The gene *acnA* encodes for the aconitase A enzyme that can catalyze Reaction 5 as citrate hydro-lyase. Figure 10 shows that transcription of *acnA* is regulated by the transcription factors Crp, FruR, MarA, Rob, SoxS, ArcA, and Fnr. These transcription factors activate or repress the promoter activity of *acnAp2* as shown in Figure 11.

![Figure 10. Transcription regulation summary diagram for the gene acnA from EcoCyc.](image)

![Figure 11. Promoter and transcription factors for the gene acnA from EcoCyc.](image)
The aconitase B enzyme that can also function as citrate hydro-lyase is encoded by the gene *acnB*. Four transcription factors (Crp, ArcA, Fis, and FruR) regulate transcription of *acnB* as illustrated by Figure 12. This gene is transcribed with the help of two promoters (*acnBp* and *acnBp2*) as shown in Figure 13. However, the transcription factors only regulate *acnBp* so the promoter *acnBp2* was not included in the model.

**Figure 12.** Transcription regulation summary diagram for the gene *acnB* from EcoCyc.

**Figure 13.** Promoters and transcription factors for the gene *acnB* from EcoCyc.

**Isocitrate Hydro-lyase:**

Aconitase A is encoded by *acnA* and can also function as the enzyme isocitrate hydro-lyase that catalyzes Reaction 6. See Figure 10 and Figure 11 for the promoter and transcription factors involved with *acnA*.

The gene *acnB* encodes for the aconitase B enzyme that can also function as isocitrate hydro-lyase. See Figure 12 for the transcription factors regulating *acnB* and see Figure 13 for the promoters involved in the transcription of *acnB*.
**Isocitrate Dehydrogenase:**

Isocitrate dehydrogenase catalyzes Reaction 7 and is encoded by the gene *icd*.

Transcription factors FruR and ArcA regulate the transcription of *icd* as illustrated by Figure 14. ArcA represses the promoter *icdAp1* and FruR activates the promoter *icdAp2* as shown in Figure 15. Both promoters, *icdAp1* and *icdAp2*, were included in the model.

![Figure 14](image1.png)

*Figure 14. Transcription regulation summary diagram for the gene *icd* from EcoCyc.*

![Figure 15](image2.png)

*Figure 15. Promoters and transcription factors for the gene *icd* from EcoCyc.*

**2-Oxoglutarate Dehydrogenase:**

The gene *sucA* encodes for the 2-oxoglutarate decarboxylase subunit of 2-oxoglutarate dehydrogenase. This enzyme catalyzes Reaction 8 in the model.

Transcription of *sucA* is regulated by Crp, Fur, ArcA, Fnr, and IHF as shown in Figure 16. The promoter *sucAp* starts the transcription of *sucA* as illustrated by Figure 17.

The gene *sucB* encodes for the second subunit of 2-oxoglutarate dehydrogenase: dihydrolipoyltranssuccinylase. The gene *sucB* is regulated by the same transcription factors and promoters as *sucA* seen in Figure 16 and Figure 17.
Figure 16. Transcription regulation summary diagram for the genes sucA, sucB, sucC, and sucD from EcoCyc.

Figure 17. Promoter and transcription factors for the genes sucA, sucB, sucC, and sucD from EcoCyc.

Lipoamide dehydrogenase is the third subunit of 2-oxoglutarate dehydrogenase and is encoded by the gene lpd. This gene is regulated by the transcription factors Fis, Crp, Fnr, Fur, ArcA, FruR, and PdhR as shown in Figure 18. The gene lpd has two promoters initiating transcription: pdhRp and lpdAp. The promoter pdhRp is affected by the transcription factors Crp, Fnr, and PdhR. The promoter lpdAp is regulated by ArcA, Crp, Fis, Fnr, and Fur as illustrated in Figure 19.
Figure 19. Promoters and transcription factors for the gene *lpd* from EcoCyc.

**Succinyl-CoA Synthetase:**

The enzyme succinyl-CoA synthetase is encoded by the genes *sucC* and *sucD*. This enzyme catalyzes Reaction 9 in the model. These genes are regulated by the same transcription factors and promoters as *sucA* and *sucB*. Figure 16 shows the transcription regulation summary diagram and Figure 17 shows the promoters used to regulate transcription of *sucC* and *sucD*.

**Succinate Dehydrogenase:**

The genes *sdhA*, *sdhB*, *sdhC*, and *sdhD* encode for the enzyme succinate dehydrogenase that catalyzes Reaction 10. These four genes are regulated by Crp, Fur, ArcA, and Fnr as seen in Figure 20. The promoters *sdhCp* and *sdhDp2* are involved in the transcription of these four genes. Figure 21 shows that the promoter *sdhCp* is regulated by ArcA, Crp, Fur, and Fnr while the promoter *sdhDp2* is only regulated by Crp. Both promoters were included in the model of the transcriptional network.

Figure 20. Transcription regulation summary diagram for the genes *sdhA*, *sdhB*, *sdhC*, and *sdhD* from EcoCyc.
Figure 21. Promoters and transcription factors for the genes *sdhA*, *sdhB*, *sdhC*, and *sdhD* from EcoCyc.

**Fumarase:**

The gene *fumA* encodes for the fumarase A enzyme that catalyzes Reaction 11. Figure 22 shows the transcription factors that regulate the transcription of *fumA*. ArcA, Crp, and Fnr regulate *fumA* through the promoter *fumAp* as shown in Figure 23.

Fumarase B is encoded by the gene *fumB* and can also catalyze Reaction 11. The transcriptional regulation of *fumB* is more complex than *fumA*. Figure 24 shows that ArcA, Crp, DcuR, Fnr, Fur, Fis, and NarL are all involved in regulating the transcription
of *fumB*. The seven transcription factors regulate the transcription of *fumB* through three promoters shown in Figure 25. Transcription factors ArcA, Fis, Fnr, and Fur are involved with the promoter *fumBp*. Transcription factors Crp, Fnr, and NarL affect the promoter *dcuBp*. The transcription factor DcuR activates promoter activity of *dcuBp2*.

Figure 24. Transcription regulation summary diagram for the gene *fumB* from EcoCyc.

The gene *fumC* encodes for fumarase C that can also catalyze Reaction 11. This gene is regulated by seven transcription factors shown in Figure 26: MarA, Rob, SoxR, SoxS, ArcA, Fnr, and Fur. These seven transcription factors regulate *fumC* through the promoter *fumCp* shown in Figure 27.
Figure 26. Transcription regulation summary diagram for the gene *fumC* from EcoCyc.

Figure 27. Promoter and transcription factors for the gene *fumC* from EcoCyc.

**Malate Dehydrogenase:**

Malate dehydrogenase catalyzes Reaction 12 and is encoded in the *E. coli* genome by the gene *mdh*. The transcription factors Crp, DpiA, ArcA, and FlhCD regulate the transcription of *mdh* as shown in Figure 28. Two promoters, *mdhp1* and *mdhp2*, are involved in the transcription of *mdh* as illustrated by Figure 29. All four transcription factors impact *mdhp1*, whereas only DpiA affects *mdhp2*.

Figure 28. Transcription regulation summary diagram for the gene *mdh* from EcoCyc.
Figure 29. Promoters and transcription factors for the gene *mdh* from EcoCyc.

**Isocitrate Lyase:**

Isocitrate lyase catalyzes Reaction 13 and is encoded by the gene *aceA*. The transcription factors FruR, IHF, ArcA, Crp, and IclR regulate the transcription of *aceA* as shown by Figure 30. These five transcription factors regulate transcription of *aceA* through their interaction with the promoter *aceBp* shown in Figure 31.

Figure 30. Transcription regulation summary diagram for the genes *aceA* and *aceB* from EcoCyc.

Figure 31. Promoter and transcription factors for the genes *aceA* and *aceB* from EcoCyc.
Malate Synthase:

Malate synthase A is the enzyme that catalyzes Reaction 14 and is encoded by the gene aceB. The transcription of aceB is under the same regulation as aceA. Figure 30 shows the five transcription factors and Figure 31 shows the promoter involved in the transcription of aceB.

Malate synthase G can also catalyze Reaction 14. This isozyme is encoded by the gene glcB. This gene is regulated by the transcription factors GlcC, IHF, and ArcA as shown in Figure 32. There are two promoters involved in the transcription of glcB: glcBp and glcDp. Figure 33 shows that only the promoter glcDp is affected by the transcription factors; therefore, only glcDp was included in the model.

Glucokinase:

The enzyme glucokinase catalyzes Reaction 15 in the model. This enzyme is encoded by the gene glk. The transcription of glk is regulated by the transcription factor
FruR as shown in Figure 34. FruR represses the promoter activity of *glkp* as shown in Figure 35. The promoter *glkp1* is not affected by transcription factors and was not included in the model.

![Figure 34](image1.png)

**Figure 34.** Transcription regulation summary diagram for the gene *glk* from EcoCyc.

![Figure 35](image2.png)

**Figure 35.** Promoters and transcription factor for the gene *glk* from EcoCyc.

**Phosphoglucomutase:**

The gene *pgm* encodes the enzyme phosphoglucomutase that catalyzes the first step in the reverse direction of Reaction 15. This gene is regulated by HU as shown in Figure 36. HU represses the activity of the *seqAp* promoter as shown in Figure 37.

![Figure 36](image3.png)

**Figure 36.** Transcription regulation summary diagram for the gene *pgm* from EcoCyc.
Glucose-1-Phosphatase:

Glucose-1-phosphatase catalyzes second step in the reverse direction of Reaction 15. This enzyme is encoded by the gene \textit{yihX}. Figure 38 and Figure 39 show that no transcription factors are known to regulate transcription of \textit{yihX}. Therefore, this gene was not included in the transcriptional network model.

Phosphoglucoisomerase:

Phosphoglucoisomerase catalyzes Reaction 16 and is encoded by the gene \textit{pgi}. Transcription of \textit{pgi} is regulated by the transcription factor SoxS as shown in Figure 40. SoxS interacts with the promoter \textit{pgip} as shown in Figure 41.
Figure 41. Promoter and transcription factor for the gene \( pgi \) from EcoCyc.

**6-Phosphofructokinase:**

The gene \( pfkA \) encodes for the enzyme 6-phosphofructokinase that catalyzes Reaction 17. This gene is regulated by the transcription factor FruR as shown in Figure 42. The promoters \( pfkAp1 \) and \( pfkAp2 \) initiate transcription of \( pfkA \) as seen in Figure 43. Only \( pfkAp2 \) interacts with FruR. Therefore, the promoter \( pfkAp1 \) was not included in the model.

Figure 42. Transcription regulation summary diagram for the gene \( pfkA \) from EcoCyc.

Figure 43. Promoters and transcription factor for the gene \( pfkA \) from EcoCyc.

The gene \( pfkB \) also encodes for 6-phosphofructokinase. However, as seen in Figure 44 and Figure 45, there are no transcription factors associated with the regulation
of pfkB. Therefore, this gene was not included in the model of the transcriptional network.

Figure 44. Transcription regulation summary diagram for the gene pfkB from EcoCyc.

Fructose-1,6-Bisphosphatase:

The enzyme fructose-1,6-bisphosphatase that catalyzes the reverse direction of Reaction 17 is encoded by the gene glpX. The transcription factors Crp and GlpR regulate the transcription of glpX shown in Figure 46. GlpR and Crp regulate the gene by interacting with the promoter glpFp shown in Figure 47.

Figure 46. Transcription regulation summary diagram for the gene glpX from EcoCyc.
Figure 47. Promoter and transcription factors for the gene \textit{glpX} from EcoCyc.

The gene \textit{ybhA} also encodes for the enzyme fructose-1,6-bisphosphatase. However, as seen in Figure 48 and Figure 49, no transcription factors or promoters are known to regulate \textit{ybhA}. Therefore, the gene \textit{ybhA} was not included in the model.

Figure 48. Transcription regulation summary diagram for the gene \textit{ybhA} from EcoCyc.

Figure 49. The gene \textit{ybhA} from EcoCyc.

Another gene, \textit{yggF}, also encodes for fructose-1,6-bisphosphatase. But, similar to the gene \textit{ybhA}, no transcription factors or promoters have been identified for \textit{yggF} as illustrated by Figure 50 and Figure 51.

Figure 50. Transcription regulation summary diagram for the gene \textit{yggF} from EcoCyc.

Figure 51. The gene \textit{yggF} from EcoCyc.
Fructose-1,6-bisphosphatase is encoded by another gene, *fbp*. Figure 52 and Figure 53 illustrate that no transcription factors or promoters are currently known to regulate *fbp*.

![Figure 52](image)

Figure 52. Transcription regulation summary diagram for the gene *fbp* from EcoCyc.

![Figure 53](image)

Figure 53. The gene *fbp* from EcoCyc.

**Fructose-Bisphosphate Aldolase:**

Fructose-bisphosphate aldolase is the enzyme that catalyzes Reaction 18 and is encoded by the gene *fbaA*. The transcription factors Crp and FruR regulate the transcription of *fbaA* as shown in Figure 54. Four promoters are known to be associated with the transcription of *fbaA*: *epdp*, *pgkp1*, *pgkp2*, and *pgkp3*. Figure 55 shows that only the promoter *epdp* is affected by the transcription factors FruR and Crp. Therefore, *epdp* was the only promoter included in the model.

Fructose-bisphosphate aldolase is also encoded by the gene *fbaB*. The gene *fbaB* is regulated by the transcription factor FruR as shown in Figure 56. FruR interacts with the promoter *fbaBp* as shown in Figure 57. The promoter *fbaBp* was included in the transcriptional network model for the enzyme fructose-bisphosphate aldolase.

![Figure 54](image)

Figure 54. Transcription regulation summary diagram for the gene *fbaA* from EcoCyc.
Figure 55. Promoters and transcription factors for the gene fbaA from EcoCyc.

Figure 56. Transcription regulation summary diagram for the gene fbaB from EcoCyc.

Figure 57. Promoter and transcription factor for the gene fbaB from EcoCyc.

**Triose Phosphate Isomerase:**

The gene tpiA encodes for the enzyme triose phosphate isomerase that catalyzes Reaction 19. The gene tpiA is regulated by the transcription factor FruR as shown in
Figure 58. There are two promoters that initiate transcription of \textit{tpiA}, but only the promoter \textit{tpiAp2} interacts with transcription factors as illustrated by Figure 59. Therefore, only the promoter \textit{tpiAp2} was included in the transcriptional network model.

![Figure 58. Transcription regulation summary diagram for the gene tpiA from EcoCyc.](image)

Figure 59. Promoters and transcription factor for the gene \textit{tpiA} from EcoCyc.

\textbf{Glyceraldehyde-3-Phosphate Dehydrogenase:}

The enzyme glyceraldehyde-3-phosphate dehydrogenase catalyzes Reaction 20 and is encoded by the gene \textit{gapA}. The transcription factors Crp and FruR regulate the transcription of \textit{gapA} as shown in Figure 60. There are four promoters for the gene \textit{gapA} as shown in Figure 61. Only the promoters \textit{gapAp1} and \textit{gapAp3} interact with Crp and FruR; therefore, only \textit{gapAp1} and \textit{gapAp3} were included in the model.

The genes \textit{gapC1} and \textit{gapC2} also encode for the enzyme glyceraldehyde-3-phosphate dehydrogenase that catalyzes Reaction 20. The transcription of these two genes is regulated by the protein Fnr as shown in Figure 62. Fnr regulates the transcription of \textit{gapC1} and \textit{gapC2} through its interaction with the promoter \textit{gapC lp} shown in Figure 63.
Figure 60. Transcription regulation summary diagram for the gene gapA from EcoCyc.

Figure 61. Promoters and transcription factors for the gene gapA from EcoCyc.

Figure 62. Transcription regulation summary diagram for the genes gapC1 and gapC2 from EcoCyc.

Figure 63. Promoter and transcription factor for the genes gapC1 and gapC2 from EcoCyc.
Phosphoglycerate Kinase:

The gene *pgk* encodes for the enzyme phosphoglycerate kinase that catalyzes Reaction 21. The transcription of *pgk* is regulated by Crp and FruR as shown in Figure 64. Four promoters initiate transcription of *pgk* as shown in Figure 65. Only the promoter *epdp* is regulated; therefore, it was the only promoter included in the model.

Figure 64. Transcription regulation summary diagram for the gene *pgk* from EcoCyc.

Figure 65. Promoters and transcription factors for the gene *pgk* from EcoCyc.
**Phosphoglycerate Mutase:**

Phosphoglycerate mutase catalyzes Reaction 22 and is encoded by the gene \( gpmM \). This gene is regulated by the transcription factor FruR as shown in Figure 66. FruR regulates transcription of \( gpmM \) through its interactions with the promoter \( gpmMp \) as shown in Figure 67.

![Figure 66. Transcription regulation summary diagram for the gene gpmM from EcoCyc.](image)

The gene \( ytjC \) also encodes for the enzyme phosphoglycerate mutase. However, as seen in Figure 68 and Figure 69, there are no known promoters and transcription factors that regulate \( ytjC \). Therefore, the gene \( ytjC \) was not included in the model.

![Figure 68. Transcription regulation summary diagram for the gene yjtc from EcoCyc.](image)

Another gene that encodes for phosphoglycerate mutase is \( gpmA \). The transcription of the gene \( gpmA \) is regulated by Fur through the promoter \( gmpAp \) as
illustrated in Figure 70 and Figure 71. Since the second promoter $gmpAp2$ does not interact with transcription factors, only the promoter $gmpAp$ was included in the model.

Figure 70. Transcription regulation summary diagram for the gene $gpmA$ from EcoCyc.

![Image](https://via.placeholder.com/150)

Figure 71. Promoters and transcription factors for the gene $gpmA$ from EcoCyc.

**Enolase:**

The gene $eno$ encodes for the enzyme enolase that catalyzes Reaction 23 in the model. The transcription of $eno$ is regulated by the transcription factor FruR as shown in Figure 72. There are nine promoters that have been identified to initiate transcription of $eno$ as shown in Figure 73. Out of the nine promoters, only the promoters $enop1$, $enop2$, and $enop3$ interact with the transcription factor FruR. Therefore, only the promoters $enop1$, $enop2$, and $enop3$ were included in the transcriptional network model.

Figure 72. Transcription regulation summary diagram for the gene $eno$ from EcoCyc.
Figure 73. Promoters and transcription factor for the gene eno from EcoCyc.
Pyruvate Kinase:

The enzyme pyruvate kinase catalyzes the forward direction of Reaction 24 and is encoded by the gene *pykF*. The transcription of *pykF* is regulated by the protein FruR as illustrated by Figure 74. The promoters *pykFp*, *pykFp1*, *pykFp2*, and *pykFp3* initiate transcription of *pykF* as shown in Figure 75. The only promoter that interacts with FruR is *pykFp*; therefore, the promoter *pykFp* was the only one included in the transcriptional network model.

Figure 74. Transcription regulation summary diagram for the gene *pykF* from EcoCyc.

Figure 75. Promoters and transcription factor for the gene *pykF* from EcoCyc.
Pyruvate kinase is also encoded by the gene *pykA*. As seen in Figure 76, there are no transcription factors known to regulate the transcription of *pykA*. Two promoters, *pykAp1* and *pykAp2*, are known to initiate transcription of *pykA* as shown in Figure 77. Since there are no transcription factors involved, the gene *pykA* was not included in the transcriptional network model.

![Figure 76. Transcription regulation summary diagram for the gene *pykA* from EcoCyc.](image)

**Phosphoenolpyruvate Synthetase:**

The gene *ppsA* encodes for the enzyme phosphoenolpyruvate synthetase that catalyzes the reverse direction of Reaction 24. Figure 78 shows that FruR is the only transcription factor that regulates *ppsA*. FruR interacts with the promoter *ppsp* as illustrated by Figure 79.

![Figure 78. Transcription regulation summary diagram for the gene *ppsA* from EcoCyc.](image)
Phosphoenolpyruvate Carboxykinase:

The gene *pck* encodes for the enzyme phosphoenolpyruvate carboxykinase that catalyzes Reaction 25. Transcription of the gene *pck* is regulated by the protein FruR as shown in Figure 80. FruR regulates transcription by interacting with the promoter *pckp* as shown in Figure 81.

Pyruvate Dehydrogenase:

The enzyme pyruvate dehydrogenase is a complex composed of three subunits. The first subunit is encoded by the gene *aceE*. Figure 82 shows that transcription of *aceE* is regulated by the proteins Crp, Fnr, ArcA, FruR, NsrR, and PdhR. The *pdhRp* and *aceEp* promoters interact with these transcription factors as illustrated by Figure 83. Both *pdhRp* and *aceEp* were included in the model.
The second subunit of pyruvate dehydrogenase is encoded by the gene \( aceF \). The gene \( aceF \) is in the same operon as the gene \( aceE \). See Figure 82 and Figure 83 for the transcription factors and promoters that regulate transcription of \( aceF \).

The gene \( lpd \) codes for the third subunit of pyruvate dehydrogenase that is also a subunit of the enzyme 2-oxoglutarate dehydrogenase. See Figure 18 and Figure 19 for the transcription factors and promoters that regulate \( lpd \).

Figure 82. Transcription regulation summary diagram for the genes \( aceE \) and \( aceF \) from EcoCyc.

Figure 83. Promoters and transcription factors for the genes \( aceE \) and \( aceF \) from EcoCyc.

Transcription Regulatory Proteins

Transcription regulatory proteins are proteins that have a DNA binding domain and regulate the transcription of genes. When the DNA binding domain is accessible, these proteins bind to the DNA near promoters. If the regulatory protein is a repressor, it interferes with the ability of the RNA polymerase to bind to the promoter and initiate
transcription. If the protein is an activator, it increases the binding affinity between RNA polymerase and the promoter in order to upregulate transcription of a gene (Lehninger et al., 2008).

Transcription factors can be turned off or on through ligand binding. Ligand binding induces a conformational shift that can either open or close the DNA binding domain of the protein (Lehninger et al., 2008). In order to correctly model the transcriptional network, it was important to understand when and how the regulatory proteins became active transcription factors. This section explains the interactions with ligands and the specific regulatory proteins identified in this model. A summary chart of the transcription factors used in this model is available in Appendix A.

**ArcA:**

ArcA is a transcriptional regulatory protein that is capable of binding to DNA when phosphorylated by the kinase ArcB. Phosphorylated ArcA acts as a repressor by binding to promoters or activator binding sites (Jeon et al., 2001). ArcA-P is a transcriptional repressor for many of the TCA cycle and glyoxylate enzymes under anaerobic conditions (Gunsalus & Park, 1994). ArcA acts as a transcriptional activator for a small number of genes by being part of a nucleoprotein complex (Sawers & Suppmann, 1992).

**Crp:**

The Crp transcriptional regulator, also known as CAP, can bind to DNA only when activated by cAMP. In the absence of glucose, cAMP-Crp levels rise allowing more Crp to bind to DNA (Ishizuka et al., 1994). Crp generally acts as a transcriptional
activator by binding upstream of the polymerase binding site. However, it can also act as a repressor by occupying the binding site of an activator protein or by interfering with the promoter region (Kolb *et al*, 1993; Liu *et al*, 2004).

**DcuR:**

The DcuR regulatory protein is activated for DNA binding through phosphorylation by kinase DcuS. Upon phosphorylation of an aspartate residue, DcuR dimerizes and is capable of binding to DNA to activate transcription (Golby *et al*, 1999).

**DpiA:**

DpiA is a transcriptional regulator that can bind to DNA when phosphorylated on an aspartate residue. It binds to DNA sequences rich in A and T nucleotides. DpiA-P is a transcriptional activator of malate dehydrogenase when citrate is available in an anaerobic environment (Yamamoto *et al*, 2008).

**Fis:**

Fis is a small protein capable of tight binding to DNA when dimerized (Finkel & Johnson, 1992). One study indicated that approximately 21% of genes are regulated by Fis (Cho *et al*, 2008). Fis requires no modifications to bind to DNA.

**FlhDC:**

FlhD and FlhC are proteins that can form the heterotetramer FlhD2C2. As a heterotetramer, FlhDC can bind to DNA and act as a transcriptional repressor or activator. No inducer has been observed for FlhDC (Stafford *et al*, 2005).
**Fn:**

Fn is a protein that activates genes needed for anaerobic metabolism and represses genes used for aerobic metabolism. The concentration of Fn remains approximately the same under aerobic and anaerobic conditions (~3.7 µM) (Sutton et al., 2004). The DNA-binding activity of Fn is regulated by oxygen. In the absence of oxygen, a [4Fe-4S] cluster binds to Fn allowing the protein to dimerize. As a dimer, Fn can bind to DNA and activate or repress transcription. In the presence of oxygen, the [4Fe-4S] cluster is oxidized into [2Fe-2S] that destabilizes the dimer (Sutton et al., 2004).

**FruR:**

FruR is a transcriptional regulator also known as Cra. Unmodified FruR can bind to DNA and can activate transcription of genes in the TCA cycle, the glyoxylate pathways, and gluconeogenesis. FruR acts as a repressor for genes involved in glycolysis. Fructose-1,6-bisphosphate and fructose-1-phosphate can bind with FruR and prevent FruR from binding to DNA (Ramseier et al., 1995).

**Fur:**

Fur is a transcriptional activator and repressor that can only bind to DNA with the cofactor Fe$^{2+}$. Fur is a regulator of many genes including some involved in the TCA cycle, glycolysis, and gluconeogenesis (Mills & Marletta, 2005).

**GlcC:**

GlcC is a dual transcriptional regulator that can bind to DNA when induced by glycolate. It regulates genes needed when glycolate is the main carbon source (Pellicer et al., 1999).
**GlpR:**

GlpR is a transcriptional repressor that can bind to DNA in its unmodified form. Binding of either glycerol or glycerol-3-phosphate to GlpR causes a conformational shift that interferes with DNA binding (Lin, 1976).

**H-NS:**

Histone-like nucleoid structuring protein (H-NS) is a small DNA-binding protein used to condense and supercoil DNA. It acts as a transcription factor for several genes, and has so far only been identified as a repressor with no inducer (Oshima et al, 2006).

**HU:**

HU is a transcriptional dual regulator. It is a small DNA-binding protein similar to histones and helps supercoil DNA. It can bind to DNA and act as a transcriptional activator or repressor without an inducer (Oberto et al, 2009).

**IclR:**

IclR is a transcriptional repressor that regulates enzymes in the glyoxylate shunt. Pyruvate and glyoxylate can bind to IclR. Pyruvate stabilizes the active tetrameric form that can bind to DNA while glyoxylate stabilizes the inactive dimer form that cannot bind to DNA (Lorca et al, 2007).

**IHF:**

IHF is a protein that is highly abundant in cells, and is used as both a transcriptional repressor and activator. IHF often stabilizes correct nucleoprotein complexes and can facilitate the formation of loops near the promoter. It is a heterodimer
protein that binds to A/T rich sequences of DNA. IHF requires no inducer to bind to DNA (Azam et al, 1999).

**LacI:**

LacI is an inducible transcriptional repressor for the lac operon. Unmodified LacI can bind to DNA at two operators forming a repressor loop to reduce transcription. Allolactose, or IPTG, can bind to LacI and cause a conformational shift that prevents LacI from binding to DNA (Lewis, 2005).

**MarA:**

MarA is a dual transcriptional regulator similar to SoxS and Rob. MarA binds to a specific DNA sequence known as the sox-mar-rob box. MarA requires no inducer to bind to DNA (Martin et al, 2008).

**NarL:**

NarL is a nitrate/nitrite response regulator that can activate and repress transcription of genes needed for nitrate respiration and other pathways. In the presence of nitrate or nitrite, the kinase NarX phosphorylates NarL. Phosphorylated NarL is capable of binding to DNA. In the absence of nitrate and nitrite, NarL is inactivated by dephosphorylation (Schröder et al, 1994).

**NsrR:**

NsrR is a nitrate-sensitive repressor that regulates at least 30 genes. NsrR can only bind to DNA with the cofactor [2Fe-2S] cluster. If nitric oxide (NO) binds to the [2Fe-
2S] cluster, then NsrR can no longer bind to DNA (Tucker et al, 2008). Ammonium nitrate is a usable nitrogen source for PHB production (Khanna & Srivastava, 2005).

**PdhR:**

PdhR is a transcriptional repressor for genes coding the pyruvate dehydrogenase and 2-oxoglutarate dehydrogenase complexes. Unmodified PdhR can bind to DNA to repress transcription. Pyruvate can bind to PdhR and cause a conformational shift that prevents PdhR from binding to target promoters (Quail & Guest, 1995).

**Rob:**

Rob is a transcriptional activator similar to SoxS and MarA and binds to the sox-mar-rob box. No inducer is needed for Rob to bind to DNA (Martin et al, 2008).

**SoxR:**

SoxR is a transcriptional activator that is activated by the cofactor [2Fe-2S] cluster. SoxR can bind to DNA with or without [2Fe-2S], but is only able to activate transcription with the presence of a [2Fe-2S] cluster (Gaudu & Weiss, 1996).

**SoxS:**

SoxS is a transcriptional activator sensitive to superoxide and nitric oxide. SoxS is similar to Rob and MarA and binds to the sox-mar-rob box DNA sequence (Martin et al, 2008). Activity of SoxS is controlled solely by its concentration (Griffith et al, 2004).
Transcriptional Network Equations

The metabolic pathways modeled consist of many enzymatic reactions. These enzymatic reactions are intrinsically dependent upon the concentration of the enzymes. The concentration of enzymes in a cell is dependent upon the quantity being transcribed and translated from the DNA coupled with the rate of enzyme degradation. Expressions were used to relate enzyme concentration to promoter activity and transcription factors. The concentration of transcription factors was related to the frequency of ligand binding. Enzyme degradation was correlated to the amino acid on the N-terminus of the enzyme’s polypeptide. The following equations were used to model the concentration of enzymes based on their transcriptional network.

**Enzyme Concentration:**

An equation was needed to correlate the amount of enzyme synthesized to its transcription factors and promoters. Equation (11) describes enzyme concentration as a function of the concentration of a single transcription factor (Ronen et al, 2002; Zaslaver et al, 2004). However, enzymes are usually regulated by multiple transcription factors.

\[
\frac{dE}{dt} = \frac{\beta}{1 + (\frac{TF}{k})^H}
\]

- \(E\) = Enzyme concentration
- \(\beta\) = Promoter activity (unrepressed/inactivated)
- \(TF\) = Transcription factor concentration
- \(k\) = Effective affinity of the transcription factor (Conc. for half maximal repression/activation)
- \(H\) = Hill coefficient of cooperativity
  - \(H > 0\) = repression
  - \(H < 0\) = activation
Equation (11) was modified as part of this thesis development to include terms for multiple transcription factors affecting one promoter. Equation (12) was the result and it relates enzyme concentration to the concentrations of $n$ transcription factors. This equation calculates the enzyme concentration based on all of the activators and repressors that interact with the promoter that initiates transcription of the enzyme’s gene.

$$\begin{equation}
\frac{dE}{dt} = \frac{\beta}{1 + \left(\frac{TF_1}{k_1}\right)^{H_1} + \left(\frac{TF_2}{k_2}\right)^{H_2} + \ldots + \left(\frac{TF_n}{k_n}\right)^{H_n}}
\end{equation}$$

- $E$ = Enzyme concentration
- $\beta$ = Promoter activity (unrepressed/inactivated)
- $TF_n$ = Transcription factor $n$ concentration
- $k_n$ = Effective affinity of transcription factor $n$ (Conc. for half maximal repression/activation)
- $H_n$ = Hill coefficient of cooperativity
  - $H > 0 = \text{repression}$
  - $H < 0 = \text{activation}$

Each promoter found to be regulated by transcription factors was represented by a reaction as shown in Figure 84. The rate for each promoter reaction was based on Equation (12). Forty promoters were used to model the transcription of 29 enzymes as seen in Figure 84. The transcription factors that regulate each promoter are shown as substrates to the promoter reactions, with enzymes shown as products of the reaction.

Eight of the promoters initiate transcription of more than one enzyme, so multiple enzymes are shown as products of those reactions. Thirteen of the enzymes are impacted by multiple promoters, so they are shown as products of multiple promoter reactions.

Table XXVII contains values from the literature that were used for the transcription factor terms in Equation (12) for each promoter reaction.
Figure 84. SimBiology model diagram of enzyme and transcription factor interactions.
Table XXVII. Transcription factor concentrations [TF] and effective affinity values ($k_n$).

<table>
<thead>
<tr>
<th>TF</th>
<th>[TF] µM</th>
<th>$k_n$</th>
<th>Organism</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>ArcA</td>
<td>NA</td>
<td>0.25-0.6</td>
<td><em>E. coli</em></td>
<td>Shen &amp; Gunsalus, 1997</td>
</tr>
<tr>
<td>Crp</td>
<td>3.3-9.4</td>
<td>NA</td>
<td><em>E. coli</em></td>
<td>Ishizuka <em>et al.</em>, 1993</td>
</tr>
<tr>
<td>DcuR</td>
<td>0.7-1.7</td>
<td>6</td>
<td><em>E. coli</em></td>
<td>Abo-Amer <em>et al.</em>, 2004</td>
</tr>
<tr>
<td>Fis</td>
<td>0.17</td>
<td>NA</td>
<td><em>E. coli</em></td>
<td>Azam <em>et al.</em>, 1999</td>
</tr>
<tr>
<td>Fnr</td>
<td>3.7</td>
<td>NA</td>
<td><em>E. coli</em></td>
<td>Sutton <em>et al.</em>, 2004</td>
</tr>
<tr>
<td>Fur</td>
<td>8.3-16.6</td>
<td>NA</td>
<td><em>E. coli</em></td>
<td>Zheng <em>et al.</em>, 1999</td>
</tr>
<tr>
<td>H-NS</td>
<td>13</td>
<td>NA</td>
<td><em>E. coli</em></td>
<td>Azam <em>et al.</em>, 1999</td>
</tr>
<tr>
<td>HU</td>
<td>50</td>
<td>NA</td>
<td><em>E. coli</em></td>
<td>Azam <em>et al.</em>, 1999</td>
</tr>
<tr>
<td>IHF</td>
<td>42</td>
<td>NA</td>
<td><em>E. coli</em></td>
<td>Azam <em>et al.</em>, 1999</td>
</tr>
<tr>
<td>LacI</td>
<td>0.033</td>
<td>NA</td>
<td><em>E. coli</em></td>
<td>Zheng <em>et al.</em>, 1999</td>
</tr>
<tr>
<td>MarA</td>
<td>0.067</td>
<td>0.02</td>
<td><em>E. coli</em></td>
<td>Martin <em>et al.</em>, 1996</td>
</tr>
<tr>
<td>NarL</td>
<td>NA</td>
<td>0.9-1.4</td>
<td><em>E. coli</em></td>
<td>Darwin <em>et al.</em>, 1997</td>
</tr>
<tr>
<td>NsrR</td>
<td>NA</td>
<td>0.02</td>
<td><em>N. gonorrhoeae</em></td>
<td>Isabella <em>et al.</em>, 2009</td>
</tr>
<tr>
<td>PdhR</td>
<td>0.61</td>
<td>0.005</td>
<td><em>E. coli</em></td>
<td>Quail &amp; Guest, 1995</td>
</tr>
<tr>
<td>Rob</td>
<td>17</td>
<td>NA</td>
<td><em>E. coli</em></td>
<td>Azam <em>et al.</em>, 1999</td>
</tr>
<tr>
<td>SoxR</td>
<td>0.17</td>
<td>0.045</td>
<td><em>E. coli</em></td>
<td>Hidalgo &amp; Demple, 1994</td>
</tr>
<tr>
<td>SoxS</td>
<td>0.2</td>
<td>0.015</td>
<td><em>E. coli</em></td>
<td>Pomposiello &amp; Demple, 2001</td>
</tr>
</tbody>
</table>

Active Transcription Factor Concentration:

Transcription factors are proteins with DNA binding domains that allow the protein to regulate transcription of certain genes. The DNA binding domain can be made more or less accessible by the binding of ligands to the transcription factor. The concentration of active transcription factor is the amount of the transcription factor that has the DNA binding domain accessible. Equation (13) shows how the fraction of bindings sites bound with a ligand is dependent upon the ligand concentration.

$$\theta = \frac{[L]}{[L]+K_d}$$

$L$ = Ligand concentration  
$K_d$ = Dissociation constant  
$\theta$ = Fraction of binding sites bound with ligand
The transcription factors FruR, GlpR, PdhR, LacI, and IclR have DNA binding domains that are made less accessible when a specific ligand binds to the transcription factor. For these transcription factors, Equation (14) was used to calculate the active transcription factor concentration in the model. In the model, Equations (13) and (14) were entered as Rules to calculate the active concentration of FruR, GlpR, PdhR, LacI, and IclR at each time step.

\[
TF_A = (1-\theta)[TF]
\]  
\[
TF_A = \text{Active transcription factor concentration} \\
\theta = \text{Fraction of binding sites bound with ligand} \\
TF = \text{Total transcription factor concentration}
\]

ArcA, CRP, DcuR, DpiA, Fnr, Fur, GlcC, IclR, NarL, NsrR, and SoxR are transcription factors that need a specific ligand bound in order to open up the DNA binding domain. Equations (13) and (15) were used to calculate the active transcription factor concentration in the model for transcription factors that require ligand-binding. Rules were used to evaluate the two equations at each time step for these 11 transcription factors.

\[
TF_A = \theta[TF]
\]  
\[
TF_A = \text{Active transcription factor concentration} \\
\theta = \text{Fraction of binding sites bound with ligand} \\
TF = \text{Total transcription factor concentration}
\]

The DNA binding domains have not yet been found to be affected by ligand-binding for the transcription factors Fis, FlhDC, H-NS, HU, IHF, MarA, Rob, and SoxS.
The active transcription factor concentration was set equal to the total transcription factor concentration for these regulatory proteins. Table XXVIII shows values found in the literature for ligand concentrations that were used in the model. The model diagram for the interactions between transcription factors and ligands is shown in Figure 85.

Table XXVIII. Known values for ligand concentrations used in the model.

<table>
<thead>
<tr>
<th>Ligand</th>
<th>[L] µM</th>
<th>Organism</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>cAMP</td>
<td>10-70</td>
<td>E. coli</td>
<td>Makman &amp; Sutherland, 1965</td>
</tr>
<tr>
<td></td>
<td>28</td>
<td>E. coli</td>
<td>Krishna et al, 2009</td>
</tr>
<tr>
<td>Glycerol</td>
<td>4200</td>
<td>S. cerevisiae</td>
<td>Hynne et al, 2001</td>
</tr>
<tr>
<td>IPTG</td>
<td>100</td>
<td>E. coli</td>
<td>Linton, 2010</td>
</tr>
<tr>
<td>P_i</td>
<td>2440</td>
<td>H. sapiens</td>
<td>Nazaret et al, 2009</td>
</tr>
<tr>
<td></td>
<td>7000</td>
<td>T. brucei</td>
<td>Helfert et al, 2001</td>
</tr>
<tr>
<td></td>
<td>6500</td>
<td>S. cerevisiae</td>
<td>Teusink et al, 2000</td>
</tr>
<tr>
<td></td>
<td>12000</td>
<td>S. cerevisiae</td>
<td>Nielsen et al, 1998</td>
</tr>
</tbody>
</table>

Figure 85. SimBiology model diagram of ligand and transcription factor interactions.
Enzyme Degradation:

The intracellular concentration of an enzyme is dependent upon the amount synthesized and degraded. Enzyme synthesis is represented in this model by the transcriptional network. Degradation reactions were added to the model to prevent constant accumulation of enzyme concentrations.

Degradation reactions were modeled using simple-mass action kinetics with the rate constant calculated from the enzyme half-life as shown in Equation (16). Enzyme half-lives were estimated based on the N-end rule (Varshavsky, 1997). Enzyme half-lives have a rough correlation with the amino acid on the N-terminus of the polypeptide chain.

\[ r = \frac{\ln(2)}{\tau} \]  

(16)

\[ r = \text{Degradation rate} \]
\[ \tau = \text{Enzyme half-life} \]

The N-terminal amino acid was determined for each enzyme and the degradation rate was calculated from the half-life determined from the N-end rule. Figure 86 shows the model diagram for the enzyme degradation reactions. Because degradation rates are only estimates, these values were adjusted later on to achieve the best fit to published models.
Figure 86. SimBiology model diagram of enzyme degradation reactions.
METABOLIC PROFILING

Introduction

Metabolic profiling is the process of monitoring the concentration of specific metabolites over time. The resulting data can be used to adjust parameters in order to create a more accurate model of the metabolic pathways in a specific strain of bacteria. Metabolic profiling requires culture samples to be quenched at specific time intervals. Quenching provides metabolic concentrations at a given time. One of the most common quenching methods is to shock the samples with a cold-buffered aqueous methanol mixture (Bajad et al, 2006; Buchholz et al, 2001; Dettmer et al, 2006; Lu et al, 2008; Mashego et al, 2007; Ohashi et al, 2007).

After sample preparations, metabolite concentrations can be determined through combinations of liquid chromatography (LC), gas chromatography (GC), mass spectrometry (MS), capillary electrophoresis (CE), and nuclear magnetic resonance (NMR) (Bajad et al, 2006; Buchholz et al, 2001; Dettmer et al, 2006; Lu et al, 2008; Mashego et al, 2007; Ohashi et al, 2007). Liquid chromatography combined with mass spectrometry (LC-MS) is effective because it can use a small sample volume to determine the concentrations of many metabolites simultaneously. One study was able to analyze the concentration of 69 metabolites from a single sample (Bajad et al, 2006).

The data obtained from metabolic profiling can be incorporated into the predictive model to allow a more precise simulation of the metabolic pathways under analysis. Metabolic profiling can continue to be used in the iterative process of genetically engineering an optimized PHB producing strain of bacteria.
Materials and Methods

**Strains and Culture Methods:**

*E. coli* XL1-Blue cells containing the pBHR68 plasmid have been shown to accumulate as much as 55% of their cell dry weight as bio-plastic (Linton, 2010). Analysis of the metabolomics was conducted on samples from this cell line and a control study was performed using untransformed *E. coli* XL1-Blue cells. The control study used the same procedures outlined below with the exception of adding ampicillin to the growth media.

Cells were pre-cultured by inoculating 5 mL of M9 growth media with 50 µL of a frozen glycerol stock. The M9 growth media was supplemented with 50 µg/mL ampicillin for selection of cells harboring the pBHR68 plasmid. Cultures were grown for 10 hours and then used to inoculate a second culture in 5 mL of M9 growth media (1:100 v/v). The second culture was grown for 10 hours and then used to inoculate a third culture in 5 mL of M9 growth media (1:100 v/v). The third culture was grown for 10 hours and then used to inoculate 150 mL M9 growth media (1:100 v/v) containing 1% (w/v) glucose and 50 µg/mL ampicillin. Cells were grown to stationary phase, and then isopropyl β-D-1-thiogalactopyranoside (IPTG) was added to a final concentration of 0.1 mM. Additional glucose, 15 mL of 7.5% (w/v) glucose solution, was added 5 hours after inoculation. Starting 5 hours after inoculation, 0.5 mL samples were taken every 15 minutes until cell growth reached stationary phase. Absorbance of the cultures was recorded at 600 nm every 30 minutes to monitor cell growth.
Quenching and Sample Preparation:

The samples were quenched for metabolic activity by adding 2.5 mL of -50°C aqueous methanol (60% v/v) containing 70 mM Hepes buffer (Bajad et al., 2006). Samples were sonicated for 2 minutes while kept on ice in order to lyse the cells. Samples were lyophilized using a speed vacuum and then re-suspended in 300 µL of quenching fluid in order to concentrate the samples. Then the samples were centrifuged for 10 minutes at 10,286g. Cell pellets were discarded and then the supernatant was stored at -80°C. All samples were stored for future analysis by high performance liquid chromatography and mass spectrometry (HPLC-MS). Recommended analytical methods for HPLC-MS are outlined in Appendix B.

Results

Growth curves obtained from the optical density for the two strains of E. coli are shown in Figure 87. After IPTG was added, the strain with the pBHR68 plasmid showed slower cell growth than the other strain as seen in Figure 87. Slower cell growth after induction by IPTG is consistent with the hypothesis that energy is being diverted from normal cellular functions in order to synthesize PHB.

Metabolic profiling data was not obtained due to the unavailability of a functioning mass-spectrometer. Metabolic profiling samples were stored at -80°C for future analysis. It was anticipated that the metabolic profiling data would be used to better fit the predictive model to the specific strain of E. coli under study. Because this data was unavailable, the model was fit to nine published models and to preliminary PHB production data as shown in the next chapter.
Figure 87. Natural log of the absorbance at time $t$ divided by (A) the initial absorbance reading and (B) absorbance reading at $t = 5$ hr plotted over time. Absorbance was read at 600 nm. At $t = 5$ hr, 15 mL of 5 x glucose solution was added to both cultures and IPTG was added to a final concentration of 0.1 mM in the XL1-Blue + pBHR68 culture. Error bars represent standard error with $n = 3$. 
The glycolysis and TCA portions of the SimBiology model were validated using published models found in the BioModels database at http://www.ebi.ac.uk/biomodels-main/publ-models.do. This database is a compilation of published models assembled by the European Bioinformatics Institute. Out of 366 curated BioModels, seven were identified as glycolysis models and two were identified as TCA cycle models. These nine models were used to validate the glycolysis pathway and the TCA cycle modeled in SimBiology.

**Glycolysis BioModels:**

Seven BioModels including 42, 61, 64, 71, 172, 176, and 177 were used to validate the glycolysis portion of the predictive model. The online simulator in the BioModels database was used to run simulations of the species in glycolysis. The SimBiology model was adjusted to match its steady-state concentrations with the steady-state concentrations of the glycolysis intermediates simulated in the BioModels. The first two BioModels (42 and 61) modeled glycolytic oscillations observed in synchronous cultures. The SimBiology model is not a model for synchronous cultures; therefore, the SimBiology model was adjusted to match the average steady-state concentrations of the glycolysis species for these two BioModels.

The SimBiology model needed to be altered to accurately match these seven BioModels. The BioModels do not account for PHB synthesis; therefore, the PHB synthesis pathway was shut off in the model by inactivating the forward and reverse
directions of Reaction 1. To account for diffusion of extracellular glucose into the cell, Reaction 0 was created using simple mass action kinetics. Reaction 27 was added to account for pyruvate used elsewhere in the cell and Reaction 28 was created to account for acetyl-CoA used in pathways not included in the model. Reactions 27 and 28 were modeled using simple mass action kinetics.

None of the BioModels accounted for the transcriptional network. Therefore, the transcriptional network portion of the SimBiology model was inactivated by holding the enzyme concentrations constant. All of the seven BioModels identified above simulated continuous cultures, so the concentration of extracellular glucose was held constant in the predictive model. The concentrations of ATP, ADP, NAD\(^+\), and NADH were also held constant during simulation. Initial concentrations of glycolysis substrates were changed to match the initial values used in each BioModel. The value zero was used for any initial concentration not specified in the BioModel. The values for Reaction 0, Reaction 27, Reaction 28, and the glycolysis enzyme concentrations were adjusted until the SimBiology simulations matched the simulations from the BioModel.

**Reaction 0:**

\[
\text{Glucose}_{\text{extracellular}} \xrightarrow{\text{Diffusion}} \text{Glucose}_{\text{intracellular}}
\]

**Reaction 27:**

\[
\text{Pyruvate} \rightarrow \text{Null}
\]

**Reaction 28:**

\[
\text{Acetyl-CoA} \rightarrow \text{Null}
\]
BioModel 42 - Nielsen 1998 Glycolysis:

BioModel 42 is a glycolysis model for *S. cerevisiae* (Nielsen *et al*, 1998). This model simulates observed oscillations in a continuous-flow, stirred tank reactor. Glycolytic oscillations in yeast are a result of a synchronous culture. BioModel 42 used the initial concentrations shown in Table XXIX. In order to fit the SimBiology model to BioModel 42, the values in Table XXIX were used as initial concentrations in the SimBiology model.

Twelve variables were adjusted in order to match the simulations between the SimBiology model and BioModel 42. Two of the variables were reaction rates for Reaction 0 and Reaction 27. The other ten variables were the glycolysis enzyme concentrations. Table XXIX shows the enzyme concentrations and reaction rates used to successfully fit the model. Figure 88 and Figure 89 visually compare the simulations from BioModel 42 with the adjusted SimBiology model.

Figure 88. Simulations from (A) BioModel 42 and (B) SimBiology model for the species glucose, fructose-6-phosphate, fructose-1,6-bisphosphate, and 1,3-diphosphoglycerate.
Figure 89. Simulations from (A) BioModel 42 and (B) SimBiology model for the species glyceraldehyde-3-phosphate, phosphoenolpyruvate, and pyruvate.

Table XXIX. Initial substrate concentrations, enzyme concentrations, and reaction rates used to compare the SimBiology model to BioModel 42.

<table>
<thead>
<tr>
<th>Species</th>
<th>Value</th>
<th>Unit</th>
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<tr>
<td>GLC_ex</td>
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<td>Nielsen et al., 1998</td>
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<tr>
<td>GLC</td>
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<td>µM</td>
<td>Nielsen et al., 1998</td>
</tr>
<tr>
<td>G6P</td>
<td>0</td>
<td>µM</td>
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<td>F6P</td>
<td>659</td>
<td>µM</td>
<td>Nielsen et al., 1998</td>
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<tr>
<td>FBP</td>
<td>7.7</td>
<td>µM</td>
<td>Nielsen et al., 1998</td>
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<td>GAP</td>
<td>1.9</td>
<td>µM</td>
<td>Nielsen et al., 1998</td>
</tr>
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<td>DHAP</td>
<td>0</td>
<td>µM</td>
<td>----</td>
</tr>
<tr>
<td>DPG</td>
<td>299</td>
<td>µM</td>
<td>Nielsen et al., 1998</td>
</tr>
<tr>
<td>3PG</td>
<td>0</td>
<td>µM</td>
<td>----</td>
</tr>
<tr>
<td>2PG</td>
<td>0</td>
<td>µM</td>
<td>----</td>
</tr>
<tr>
<td>PEP</td>
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<td>µM</td>
<td>Nielsen et al., 1998</td>
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<td>PYR</td>
<td>4.2</td>
<td>µM</td>
<td>Nielsen et al., 1998</td>
</tr>
<tr>
<td>KIN</td>
<td>310</td>
<td>µM</td>
<td>----</td>
</tr>
<tr>
<td>PGI</td>
<td>50</td>
<td>µM</td>
<td>----</td>
</tr>
<tr>
<td>6PFK</td>
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<td>----</td>
</tr>
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<td>TPI</td>
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<td>PGM</td>
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<td>µM</td>
<td>----</td>
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<tr>
<td>PYK</td>
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<td>µM</td>
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<tr>
<td>Reaction Rates</td>
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</tr>
<tr>
<td>R0</td>
<td>0.01</td>
<td>µM/s</td>
<td>----</td>
</tr>
<tr>
<td>R27</td>
<td>1.17</td>
<td>µM/s</td>
<td>----</td>
</tr>
</tbody>
</table>
BioModel 61 - Hynne 2001 Glycolysis:

BioModel 61 is another model for glycolysis in *S. cerevisiae* (Hynne *et al.*, 2001). Similar to BioModel 42, this model simulates glycolytic oscillations in yeast. BioModel 61 used the initial species concentrations found in Table XXX. The values in Table XXX were used as initial substrate concentrations to fit the SimBiology model to BioModel 61.

Many different values for ten enzymes and two reaction rates were tried in order to successfully fit the model to BioModel 61. Table XXX shows the combination of values that resulted in a successful fit to BioModel 61. The matching simulations between the model and BioModel 61 are shown in Figure 90, Figure 91, and Figure 92.

BioModel 61 did not have simulations available for the species 2-phosphoglycerate and 3-phosphoglycerate. The average simulation value from the other BioModels for 2-phosphoglycerate was 0.062 mM. The average simulation value for 3-phosphoglycerate was 0.48 mM. For these two species, the SimBiology model was fit to match the average values as shown in Figure 93.

![Figure 90](image1.png)

Figure 90. Simulations from (A) BioModel 61 and (B) SimBiology model for the species glucose intracellular, glucose extracellular, and pyruvate.
Figure 91. Simulations from (A) BioModel 61 and (B) SimBiology model for the species dihydroxyacetone phosphate, fructose-1,6-bisphosphate, fructose-6-phosphate, and glucose-6-phosphate.

Figure 92. Simulations from (A) BioModel 61 and (B) SimBiology model for the species 1,3-diphosphoglycerate, glyceraldehyde-3-phosphate, and phosphoenolpyruvate.

BioModel 64 – Teusink 2000 Glycolysis:

BioModel 64 is another glycolysis model for *S. cerevisiae* (Teusink *et al*, 2000). This model simulates steady-state fluxes in a continuous fed reactor. Unlike BioModel 42 and 61, this BioModel does not model glycolytic oscillations. The initial species concentrations in Table XXXI were used in BioModel 64 and in the SimBiology model.
Figure 93. Simulation from SimBiology model for the species 2-phosphoglycerate and 3-phosphoglycerate. BioModel 61 had no simulations for these species.

Table XXX. Initial substrate concentrations, enzyme concentrations, and reaction rates used to compare the SimBiology model to BioModel 61.

<table>
<thead>
<tr>
<th>Species</th>
<th>Value</th>
<th>Unit</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>GLC_ex</td>
<td>24000</td>
<td>µM</td>
<td>Hynne et al, 2001</td>
</tr>
<tr>
<td>GLC</td>
<td>573</td>
<td>µM</td>
<td>Hynne et al, 2001</td>
</tr>
<tr>
<td>G6P</td>
<td>4200</td>
<td>µM</td>
<td>Hynne et al, 2001</td>
</tr>
<tr>
<td>F6P</td>
<td>490</td>
<td>µM</td>
<td>Hynne et al, 2001</td>
</tr>
<tr>
<td>FBP</td>
<td>4640</td>
<td>µM</td>
<td>Hynne et al, 2001</td>
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<tr>
<td>GAP</td>
<td>115</td>
<td>µM</td>
<td>Hynne et al, 2001</td>
</tr>
<tr>
<td>DHAP</td>
<td>2950</td>
<td>µM</td>
<td>Hynne et al, 2001</td>
</tr>
<tr>
<td>DPG</td>
<td>0.27</td>
<td>µM</td>
<td>Hynne et al, 2001</td>
</tr>
<tr>
<td>3PG</td>
<td>0</td>
<td>µM</td>
<td>----</td>
</tr>
<tr>
<td>2PG</td>
<td>0</td>
<td>µM</td>
<td>----</td>
</tr>
<tr>
<td>PEP</td>
<td>40</td>
<td>µM</td>
<td>Hynne et al, 2001</td>
</tr>
<tr>
<td>PYR</td>
<td>8700</td>
<td>µM</td>
<td>Hynne et al, 2001</td>
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<tr>
<td>KIN</td>
<td>310</td>
<td>µM</td>
<td>----</td>
</tr>
<tr>
<td>PGI</td>
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<td>µM</td>
<td>----</td>
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<tr>
<td>6PFK</td>
<td>4.0</td>
<td>µM</td>
<td>----</td>
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<td>FBA</td>
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<td>TPI</td>
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<td>GAD</td>
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<td>µM</td>
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<td>PGM</td>
<td>2.06</td>
<td>µM</td>
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<td>ENO</td>
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<td>PYK</td>
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<td>R0</td>
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<td>µM/s</td>
<td>----</td>
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<tr>
<td>R27</td>
<td>0.022</td>
<td>µM/s</td>
<td>----</td>
</tr>
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</table>
Different combinations of enzyme concentrations and reaction rate values were simulated in the SimBiology model. The values found in Table XXXI were a successful combination that resulted in a good fit to BioModel 64. Figure 94 and Figure 95 show how closely the SimBiology model simulations matched the BioModel 64 simulations.

BioModel 64 had no simulations available for glyceraldehyde-3-phosphate and dihydroxyacetone phosphate. The average concentration for glyceraldehyde-3-phosphate from other BioModels was 2.14 mM. The average concentration for dihydroxyacetone phosphate from BioModels 71, 172, 176, and 177 was 0.032 mM. The SimBiology model was adjusted to match these average values as shown in Figure 96.

![Figure 94](image)

Figure 94. Simulations from (A) BioModel 64 and (B) SimBiology model for the species 3-phosphoglycerate, fructose-1,6-bisphosphate, glucose-6-phosphate, and pyruvate.

**BioModel 71 - Bakker 2001 Glycolysis:**

BioModel 71 simulates glycolysis in the protist species *T. brucei* (Helfert et al., 2001). Like the other glycolysis BioModels, this one also models a continuous fed reactor. Initial substrate concentrations in the SimBiology model were set to match the initial concentrations in BioModel 71 shown in Table XXXII.
Figure 95. Simulations from BioModel 64 for the species 1,3-diphosphoglycerate, 2-phosphoglycerate, fructose-6-phosphate, glucose intracellular, and phosphoenolpyruvate.

Table XXXI. Initial substrate concentrations, enzyme concentrations, and reaction rates used to compare the SimBiology model to BioModel 64.

<table>
<thead>
<tr>
<th>Species</th>
<th>Value</th>
<th>Unit</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>GLC_ex</td>
<td>50000</td>
<td>µM</td>
<td>Teusink et al, 2000</td>
</tr>
<tr>
<td>GLC</td>
<td>87</td>
<td>µM</td>
<td>Teusink et al, 2000</td>
</tr>
<tr>
<td>G6P</td>
<td>2450</td>
<td>µM</td>
<td>Teusink et al, 2000</td>
</tr>
<tr>
<td>F6P</td>
<td>620</td>
<td>µM</td>
<td>Teusink et al, 2000</td>
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<tr>
<td>FBP</td>
<td>5510</td>
<td>µM</td>
<td>Teusink et al, 2000</td>
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<tr>
<td>GAP</td>
<td>0</td>
<td>µM</td>
<td>----</td>
</tr>
<tr>
<td>DHAP</td>
<td>0</td>
<td>µM</td>
<td>----</td>
</tr>
<tr>
<td>DPG</td>
<td>0</td>
<td>µM</td>
<td>----</td>
</tr>
<tr>
<td>3PG</td>
<td>900</td>
<td>µM</td>
<td>Teusink et al, 2000</td>
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<td>2PG</td>
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<td>µM</td>
<td>Teusink et al, 2000</td>
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<tr>
<td>PEP</td>
<td>70</td>
<td>µM</td>
<td>Teusink et al, 2000</td>
</tr>
<tr>
<td>PYR</td>
<td>1850</td>
<td>µM</td>
<td>Teusink et al, 2000</td>
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<td>6PFK</td>
<td>1.05</td>
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<td>----</td>
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<td>FBA</td>
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<td>µM</td>
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<td>TPI</td>
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<td>µM</td>
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</tr>
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<td>PGK</td>
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<td>µM</td>
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<td>µM</td>
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<td>ENO</td>
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</tr>
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<td>PYK</td>
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<td>R0</td>
<td>0.0008</td>
<td>µM/s</td>
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<tr>
<td>R27</td>
<td>0.00799</td>
<td>µM/s</td>
<td>----</td>
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</table>
The values of the glycolysis enzymes and the reaction rates of Reaction 0 and Reaction 27 were adjusted to fit the model to BioModel 71. The values that resulted in a successful fit are found in Table XXXII. The best fit was determined by comparing each glycolysis substrate simulation between BioModel 71 and the SimBiology model. Figure 97, Figure 98, and Figure 99 show how the simulations of BioModel 71 compare with the simulations of the SimBiology model.
Table XXXII. Initial substrate concentrations, enzyme concentrations, and reaction rates used to compare the SimBiology model to BioModel 71.

<table>
<thead>
<tr>
<th>Species</th>
<th>Value</th>
<th>Unit</th>
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<tbody>
<tr>
<td>GLC_ex</td>
<td>50000</td>
<td>µM</td>
<td>Helfert et al, 2001</td>
</tr>
<tr>
<td>GLC</td>
<td>34</td>
<td>µM</td>
<td>Helfert et al, 2001</td>
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<td>G6P</td>
<td>2072</td>
<td>µM</td>
<td>Helfert et al, 2001</td>
</tr>
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<td>F6P</td>
<td>512</td>
<td>µM</td>
<td>Helfert et al, 2001</td>
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<td>16537</td>
<td>µM</td>
<td>Helfert et al, 2001</td>
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<td>39.9</td>
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<td>3899</td>
<td>µM</td>
<td>Helfert et al, 2001</td>
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<td>µM</td>
<td>Helfert et al, 2001</td>
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<td>3PG</td>
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<td>µM</td>
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</tr>
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<td>2PG</td>
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<td>----</td>
</tr>
<tr>
<td>PEP</td>
<td>0</td>
<td>µM</td>
<td>Helfert et al, 2001</td>
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<td>4774</td>
<td>µM</td>
<td>Helfert et al, 2001</td>
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<tr>
<td>PYK</td>
<td>1.2015</td>
<td>µM</td>
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<table>
<thead>
<tr>
<th>Reaction Rates Used to Fit Model</th>
<th>Value</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>R0</td>
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<td>µM/s</td>
</tr>
<tr>
<td>R27</td>
<td>0.01846</td>
<td>µM/s</td>
</tr>
</tbody>
</table>

Figure 98. Simulations from (A) BioModel 71 and (B) SimBiology model for the species 3-phosphoglycerate, fructose-6-phosphate, and phosphoenolpyruvate.
BioModel 71 had no simulation available for 2-phosphoglycerate. The SimBiology simulation of 2-phosphoglycerate was fitted to the average concentration from the other BioModels. Figure 100 shows how the simulation matches the average value of 0.62 mM for 2-phosphoglycerate.
BioModel 172 - Pritchard 2002 Glycolysis:

BioModel 172 is a model of *S. cerevisiae* glycolysis (Pritchard & Kell, 2002). Similar to BioModel 64, this model does not simulate glycolytic oscillations in yeast. BioModel 172 is a model for a continuous fed reactor. Initial species concentrations used in BioModel 172 were also used in the SimBiology model and are shown in Table XXXIII. Enzyme concentrations and reaction rates were varied in the SimBiology model in order to find a successful fit to BioModel 172. The best fit came from the values in Table XXXIII.

Comparison of species simulations between BioModel 172 and the SimBiology model are shown in Figure 101, Figure 102, and Figure 103. These figures show closely matched simulations for all 11 glycolysis species that BioModel 172 can simulate.

![Figure 101](image1.png)

Figure 101. Simulations from (A) BioModel 172 and (B) SimBiology model for the species fructose-1,6-bisphosphate, extracellular glucose, glucose-6-phosphate, and pyruvate.
Figure 102. Simulations from (A) BioModel 172 and (B) SimBiology model for the species fructose-6-phosphate, intracellular glucose, 2-phosphoglycerate, and 3-phosphoglycerate.

Figure 103. Simulations from (A) BioModel 172 and (B) SimBiology model for the species glyceraldehyde-3-phosphate, 1,3-diphosphoglycerate, and phosphoenolpyruvate.

BioModel 172 had no simulation available for dihydroxyacetone phosphate. The simulation for dihydroxyacetone phosphate in the SimBiology model was matched to an average concentration from other BioModels. BioModels 176 and 177 had an average concentration of 0.785 mM for dihydroxyacetone phosphate. Figure 104 shows the SimBiology simulation of dihydroxyacetone phosphate to be close to this value.
Table XXXIII. Initial substrate concentrations, enzyme concentrations, and reaction rates used to compare the SimBiology model to BioModel 172.

<table>
<thead>
<tr>
<th>Species</th>
<th>Value</th>
<th>Unit</th>
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<td>GLC_ex</td>
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<td>µM</td>
<td>Pritchard &amp; Kell, 2002</td>
</tr>
<tr>
<td>GLC</td>
<td>97.7</td>
<td>µM</td>
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</tr>
<tr>
<td>G6P</td>
<td>2675</td>
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<tr>
<td>F6P</td>
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Figure 104. Simulation from SimBiology model for the species dihydroxyacetone phosphate. BioModel 172 had no simulations available for this species.
BioModel 176 - Conant 2007 WGD Glycolysis 2A3AB:

BioModel 176 is also a simulation of glycolysis in the species S. cerevisiae (Conant & Wolfe, 2007). This BioModel is based on a continuous fed reactor and does not model glycolytic oscillations in yeast. Table XXXIV shows the initial species concentrations from BioModel 176 that were also used in the SimBiology model. The best fit to BioModel 176 was achieved by adjusting the twelve variables shown in Table XXXIV until the SimBiology simulations matched the simulations from BioModel 176.

BioModel 176 had simulations available for all of the glycolysis species. The best fit to BioModel 176 used the values found in Table XXXIV for the enzyme concentrations and reaction rates. The simulations from this best fit are shown next to the simulations from BioModel 176 in Figure 105 and Figure 106.

Figure 105. Simulations from (A) BioModel 176 and (B) SimBiology model for the species glucose-6-phosphate, fructose-6-phosphate, fructose-1,6-bisphosphate, dihydroxyacetone phosphate, 3-phosphoglycerate, and pyruvate.
Figure 106. Simulations from (A) BioModel 176 and (B) SimBiolo
gy model for the species intracellular glucose, glyceraldehyde-3-
phosphate, 1,3-diphosphoglycerate, 2-
phosphoglycerate, and phosphoenolpyruvate.

Table XXXIV. Initial substrate concentrations, enzyme concentrations, and reaction rates
used to compare the SimBiolo
gy model to BioModel 176.

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<td>µM</td>
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BioModel 177 - Conant 2007 Glycolysis 2C:

BioModel 177 is based on the same study for *S. cerevisiae* as BioModel 176 (Conant & Wolfe, 2007). The main difference between models is that BioModel 177 includes acetyl-CoA synthesis from pyruvate. This is the only glycolysis BioModel that simulates acetyl-CoA. In order to fit simulations of acetyl-CoA to the BioModel, Reaction 28 was added to account for acetyl-CoA that is used elsewhere in the cell. Reaction 28 uses simple mass action kinetics, and the rate value was adjusted to achieve the best fit to the simulations from BioModel 177.

Initial substrate concentrations in the SimBiology model were set equal to initial concentrations used in BioModel 177. These values are shown in Table XXXV. The best fit to BioModel 177 was found by varying the values for the ten glycolysis enzymes and the three reaction rates for Reaction 0, Reaction 27, and Reaction 28. The values that resulted in the best fit are shown in Table XXXV.

Simulations for each glycolysis substrate and acetyl-CoA were compared between the SimBiology model and BioModel 177. The simulation values of acetyl-CoA, pyruvate, and fructose-1,6-bisphosphate were very similar between the models as shown in Figure 107. Comparable values were seen between the models for the rest of the species as shown in Figure 108, Figure 109, and Figure 110.

Target Values in Glycolysis for the SimBiology Model:

Comparisons with the seven glycolysis BioModels resulted in a distribution of enzyme concentrations, reaction rates, and initial species concentrations that could be used in the final SimBiology model. Figure 111 shows box plots representing the range of values used for the initial species concentrations in glycolysis. Ten outliers out of 84
values were identified in these box plots. Outliers were removed and then an average value was calculated for each species. Table XXXVI shows the resulting averages that were used in the final SimBiology model for initial species concentrations.

The distribution of rate values for Reaction 0 and Reaction 27 are shown by the box plots in Figure 112. One outlier out of 14 values was identified and removed before average values were calculated. Table XXXVI shows the resulting averages that were used as reaction rates in the final SimBiology model.

Table XXXV. Initial substrate concentrations, enzyme concentrations, and reaction rates used to compare the SimBiology model to BioModel 177.

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<tr>
<td>GLC</td>
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<td>µM</td>
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Figure 107. Simulations from (A) BioModel 177 and (B) SimBiology model for the species fructose-1,6-bisphosphate, pyruvate, and acetyl-CoA.

Figure 108. Simulations from (A) BioModel 177 and (B) SimBiology model for the species intracellular glucose, glucose-6-phosphate, fructose-6-phosphate, dihydroxyacetone phosphate, and 3-phosphoglycerate.

Figure 109. Simulations from (A) BioModel 177 and (B) SimBiology model for the species 1,3-diphosphoglycerate, 2-phosphoglycerate, and phosphoenolpyruvate.
Figure 110. Simulations from (A) BioModel 177 and (B) SimBiology model for the species glyceraldehyde-3-phosphate.

Figure 111. Box plots showing the distribution of initial values for (A) glucose, glyceraldehyde-3-phosphate, 1,3-diphosphoglycerate, 2-phosphoglycerate, phosphoenolpyruvate, (B) glucose-6-phosphate, fructose-1,6-bisphosphate, dihydroxyacetone phosphate, pyruvate, (C) fructose 6-phosphate, and 3-phosphoglycerate used in the glycolysis BioModels. Ten outliers out of 84 values were identified.
Figure 112. Box plots showing the distribution of rates for (A) Reaction 0 and (B) Reaction 27 used to fit the seven glycolysis BioModels. One outlier out of 14 values was identified from these box plots.

Table XXXVI. Initial substrate concentrations, target enzyme concentrations, and reaction rates used for the final model based on the seven glycolysis BioModels. Values are averages of the seven BioModels once outliers have been removed.

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Distributions of the glycolysis enzyme values are shown in the box plots in Figure 113. Seven outliers out of 70 values were identified in the box plots. The outliers were removed, and then average enzyme values were calculated. The average enzyme values are shown in Table XXXVI and were used as target values in the model.

Figure 113. Box plots showing the distribution of values for the enzymes (A) kinase, fructose-bisphosphate aldolase, glyceraldehyde-3-phosphate dehydrogenase, enolase (B) 6-phosphofructokinase, triose phosphate isomerase, phosphoglycerate mutase, pyruvate kinase (C) phosphoglucone isomerase, and phosphoglycerate kinase used to fit the seven glycolysis BioModels. Seven outliers out of 70 values were identified in these box plots.

Due to the presence of the transcriptional network, enzyme concentrations were not held constant in the final model. Initial enzyme concentrations in the final model were set equal to the values in Table XXXVI. Enzyme concentrations during the
simulation are combination of the synthesis and degradation of the enzymes. Each enzyme was monitored during simulations, and then promoter activity and enzyme degradation rates were adjusted in order to achieve enzyme concentrations close to the target values shown in Table XXXVI.

**TCA Cycle BioModels:**

The BioModels 222 and 232 were used to validate the TCA cycle in the predictive model. The online simulator in the BioModels database was used to create simulations of the substrates in the TCA cycle. Values in the SimBiology model were adjusted to mimic the simulations of each BioModel.

The BioModels 222 and 232 do not account for glycolysis, acetyl-CoA synthesis, or PHB synthesis. In the SimBiology model, these four pathways were shut off by inactivating the forward and reverse directions of Reaction 1, Reaction 13, Reaction 14, and Reaction 26. The concentration of each enzyme was held constant in order to inactivate the transcriptional network portion of the model. The concentration of acetyl-CoA was held constant in both BioModels and in the SimBiology model. The concentrations of CoA, ATP, ADP, NAD\(^+\), and NADH were also held constant during simulation. Initial concentrations of substrates in the TCA cycle were changed to match the initial values used in each BioModel. The values for TCA cycle enzyme concentrations were adjusted until the SimBiology simulations matched the simulations from the BioModel.
BioModel 222 - Singh 2006 TCA Cycle:

BioModel 222 is the only BioModel that simulates the TCA cycle in *E. coli* (Singh & Ghosh, 2006). BioModel 222 includes the glyoxylate bypass in the TCA cycle and has simulations available for ten species in the TCA cycle. BioModel 222 held acetyl-CoA constant during simulations, so acetyl-CoA was also held constant in the SimBiology model. Initial species concentrations in the SimBiology model were set equal to the values used in BioModel 222 that are shown in Table XXXVII. A best fit was determined by adjusting the TCA cycle enzyme concentrations and comparing simulations.

Table XXXVII. Initial substrate concentrations and enzyme concentrations used to compare the SimBiology model to BioModel 222.

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<td>3.7</td>
<td>µM</td>
<td>-----</td>
</tr>
<tr>
<td>IL</td>
<td>0.1</td>
<td>µM</td>
<td>-----</td>
</tr>
<tr>
<td>MS</td>
<td>10</td>
<td>µM</td>
<td>-----</td>
</tr>
</tbody>
</table>
Simulations for each TCA cycle species closely matched the simulations from BioModel 222 when the enzyme concentrations in Table XXXVII were used. Figure 114 visually compares the values for acetyl-CoA, malate, fumarate, succinyl-CoA, and succinate between BioModel 222 and the SimBiology model. Figure 115 illustrates the closely matched values for oxaloacetate, 2-oxoglutarate, and isocitrate between the two models. Figure 116 shows the comparison between the last two species: citrate and glyoxylate.

![Figure 114. Simulations from (A) BioModel 222 and (B) SimBiology model for the species acetyl-CoA, succinyl-CoA, succinate, fumarate, and malate.](image1)

![Figure 115. Simulations from (A) BioModel 222 and (B) SimBiology model for the species oxaloacetate, isocitrate, and 2-oxoglutarate.](image2)
Figure 116. Simulations from (A) BioModel 222 and (B) SimBiology model for the species citrate and glyoxylate.

**BioModel 232 - Nazaret 2009 TCA Cycle:**

BioModel 232 was the only other BioModel found for the TCA cycle. The model simulates the TCA cycle in *Homo sapiens* (Nazaret *et al*, 2009). This BioModel was not the most desirable to use because it does not model the TCA cycle in a microorganism and it only simulates four of the TCA cycle species. BioModel 232 also does not include the glyoxylate bypass. However, since only two BioModels simulate the TCA cycle, it was decided to try and fit the SimBiology model to BioModel 232. Table XXXVIII shows the initial species concentrations used in BioModel 232 that were also used in the SimBiology model. BioModel 232 did not have initial values for five of the TCA cycle species, so initial values from BioModel 222 were used.

The TCA cycle enzyme concentrations were varied in the SimBiology model in order to find the best fit to BioModel 232. The values of isocitrate lyase and malate synthase were automatically set to zero since BioModel 232 did not include the glyoxylate bypass. However, only three of the enzymes could be confidently adjusted and fit to BioModel 232 since the model only had simulations available for acetyl-CoA,
oxaloacetate, citrate, and 2-oxoglutarate. Table XXXVIII shows the values used to fit the SimBiology model to BioModel 232. The values for citrate synthase, citrate hydro-lyase, and 2-oxoglutarate dehydrogenase were the key to matching the simulations of the four TCA cycle species between models. Figure 117 illustrates the fit between the SimBiology model and BioModel 232 based on four of the TCA cycle species.

Table XXXVIII. Initial substrate concentrations and enzyme concentrations used to compare the SimBiology model to BioModel 232.

<table>
<thead>
<tr>
<th>Species</th>
<th>Value</th>
<th>Unit</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ac-CoA</td>
<td>63</td>
<td>µM</td>
<td>Nazaret et al, 2009</td>
</tr>
<tr>
<td>OAA</td>
<td>5</td>
<td>µM</td>
<td>Nazaret et al, 2009</td>
</tr>
<tr>
<td>CIT</td>
<td>440</td>
<td>µM</td>
<td>Nazaret et al, 2009</td>
</tr>
<tr>
<td>ISO</td>
<td>18</td>
<td>µM</td>
<td>Singh &amp; Ghosh, 2006</td>
</tr>
<tr>
<td>2-OXO</td>
<td>225</td>
<td>µM</td>
<td>Nazaret et al, 2009</td>
</tr>
<tr>
<td>SCA</td>
<td>40</td>
<td>µM</td>
<td>Singh &amp; Ghosh, 2006</td>
</tr>
<tr>
<td>SUC</td>
<td>600</td>
<td>µM</td>
<td>Singh &amp; Ghosh, 2006</td>
</tr>
<tr>
<td>FUMA</td>
<td>300</td>
<td>µM</td>
<td>Singh &amp; Ghosh, 2006</td>
</tr>
<tr>
<td>MAL</td>
<td>1800</td>
<td>µM</td>
<td>Singh &amp; Ghosh, 2006</td>
</tr>
<tr>
<td>CS</td>
<td>18.0</td>
<td>µM</td>
<td>----</td>
</tr>
<tr>
<td>CHL</td>
<td>44.5</td>
<td>µM</td>
<td>----</td>
</tr>
<tr>
<td>ICHL</td>
<td>1.0</td>
<td>µM</td>
<td>----</td>
</tr>
<tr>
<td>ICDH</td>
<td>1.0</td>
<td>µM</td>
<td>----</td>
</tr>
<tr>
<td>OGDH</td>
<td>1770.4</td>
<td>µM</td>
<td>----</td>
</tr>
<tr>
<td>SCS</td>
<td>50.0</td>
<td>µM</td>
<td>----</td>
</tr>
<tr>
<td>SDH</td>
<td>50.0</td>
<td>µM</td>
<td>----</td>
</tr>
<tr>
<td>FUM</td>
<td>50.0</td>
<td>µM</td>
<td>----</td>
</tr>
<tr>
<td>MDH</td>
<td>50.0</td>
<td>µM</td>
<td>----</td>
</tr>
<tr>
<td>IL</td>
<td>0</td>
<td>µM</td>
<td>----</td>
</tr>
<tr>
<td>MS</td>
<td>0</td>
<td>µM</td>
<td>----</td>
</tr>
</tbody>
</table>

Target Values in the TCA Cycle for the SimBiology Model:

For the final SimBiology model, it was decided to use the initial species concentrations in Table XXXVII that were used in BioModel 222. These values were chosen because BioModel 222 was specifically for E. coli and had values for each TCA cycle species. Initial enzyme concentrations were set to the values in Table XXXVII. In
the final model, promoter activity and enzyme degradation rates were adjusted to simulate enzyme concentrations consistent with the target values in Table XXXVII.

![Figure 117. Simulations from (A) BioModel 232 and (B) SimBiology model for the species acetyl-CoA, 2-oxoglutarate, citrate, and oxaloacetate.]

Comparing Model with Experimental Data

No BioModels were available for the synthesis of PHB. The PHB synthesis pathway in the model was validated using experimental data for PHB production. Table XXXIX contains preliminary experimental PHB production data for two strains of *E. coli*. Each strain contains the genes for the PHB synthesis pathway, but they are carried in different plasmids. The SimBiology model was built specifically for *E. coli* carrying the pBHR68 plasmid. The data for the 4MHT plasmid was included to demonstrate that the predictive model is capable of being fitted to a specific strain of bacteria.

The enzymes in the PHB synthesis pathway (PhaA, PhaB, and PhaC) were held constant in order to fit the model to the data. Different enzyme concentrations were tried until the simulated PHB closely matched the PHB production data. Figure 118 shows the
simulations that best fit the PHB production data for the two strains. Table XL shows the enzyme concentrations used to achieve these two fits.

Table XXXIX. Experimental data for PHB production in *E. coli* strains containing 4MHT or pBHR68 plasmids.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Time (hrs)</th>
<th>PHB (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4MHT*</td>
<td>8</td>
<td>0.0066</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>0.0262</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>0.4406</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>0.4609</td>
</tr>
<tr>
<td>pBHR68</td>
<td>8</td>
<td>Not detected</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>0.0348</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>0.0604</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>0.1120</td>
</tr>
</tbody>
</table>

*Plasmid 4MHT consists of the pBHR68 genes, the phasin gene *phaP1*, and the membrane protein gene *hlyA*.

Figure 118. Simulated PHB fit to the PHB production data from *E. coli* strains with (A) the 4MHT plasmid and (B) the pBHR68 plasmid.

In the final SimBiology model, the enzyme values were not held constant. Promoter activity and enzyme degradation rates were adjusted to generate the desired enzyme concentrations. The concentrations in Table XL for the pBHR68 data were the target values used since the model was created for the pBHR68 strain. Experimental data from metabolic profiling can be used in the same way as the PHB production data. A
better predictive model for a specific microorganism can be created by fitting the model to more experimental data from that specific strain. A tutorial on how to download and use the final model is available in Appendix D.

Table XL. Enzyme concentrations used to fit the model to experimental PHB production data.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Enzyme</th>
<th>Concentration (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4MHT</td>
<td>PhaA</td>
<td>110</td>
</tr>
<tr>
<td></td>
<td>PhaB</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>PhaC</td>
<td>5</td>
</tr>
<tr>
<td>pBHR68</td>
<td>PhaA</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td>PhaB</td>
<td>0.41</td>
</tr>
<tr>
<td></td>
<td>PhaC</td>
<td>0.5</td>
</tr>
</tbody>
</table>

*Plasmid 4MHT consists of the pBHR68 genes, the phasin gene *phaP1*, and the membrane protein gene *hlyA*.

Model Optimization and Results

The final SimBiology model was used to identify target genes and promoters for genetic engineering. In the model, the value $K_m$ is a quantitative measure of the binding affinity an enzyme has with its substrate. Lower values of $K_m$ represent higher substrate affinity while higher values of $K_m$ represent lower substrate affinity. An enzyme’s substrate affinity is dependent upon the amino acids that interact with the substrate in the enzyme’s active site. These amino acids can be identified in the gene and then site-directed mutagenesis can be used to change one or more of these amino acids. Changing the amino acids of the active site can increase or decrease the enzyme’s substrate affinity depending upon the new amino acids used. However, it is easier to make an enzyme less effective at binding a substrate than trying to make it more effective.
In the final SimBiology model, $K_m$ values for each enzyme were adjusted and simulations were conducted to determine if the changes resulted in an increase in PHB production. Adjusting the $K_m$ values resulted in no visible increase in PHB production for 16 enzymes, a slight increase in PHB production for 5 enzymes, and a large increase in PHB production for 9 enzymes.

The transcriptional network portion of the model was also adjusted to optimize the model for increased PHB production. Most promoters, transcription factors, and ligands were not adjusted for optimization because of their wide use in metabolic pathways not included in the model. Adjustments were conducted on the transcriptional network directly associated with the pBHR68 plasmid that encodes the genes of the PHB synthesis pathway.

**Adjusting Enzyme Substrate Affinity:**

The $K_m$ values for each of the 30 enzymes in this model were increased up to five times or decreased down to a fifth of the original value. These values were used to standardize comparisons of increased PHB production between enzymes. This is roughly the same as making the enzyme active site five times less effective at binding the substrate or five times more effective at binding the substrate. The direction of adjustment that resulted in an increase in PHB production was the simulation kept for comparison. Simulations were compared between enzymes to determine which adjustments resulted in the greatest increase in PHB production.

**PHB Synthesis Enzymes:**

Decreasing the $K_m$ value for β-ketothiolase (PhaA) increased the PHB production as shown in Figure 119. Decreasing the $K_m$ by one-fifth resulted in a 35.3% increase in
PHB production. If \( \beta \)-ketothiolase has a better binding affinity then it results in higher synthesis of PHB.

The enzyme acetoacetyl-CoA reductase (PhaB) has a \( K_m \) value for the substrate acetoacetyl-CoA and the substrate NADPH. A five-fold decrease in the \( K_m \) value for acetoacetyl-CoA resulted in a 1.0% increase in the rate of PHB production, and decreasing the \( K_m \) for NADPH by a fifth resulted in a 7.6% increase in the rate of PHB production as seen Figure 120. By decreasing both \( K_m \) values, an 8.6% increase in the rate of production was observed as seen in Figure 121.

Figure 119. PHB production levels from adjusting PhaA’s \( K_m \) for acetyl-CoA.

Figure 120. PHB production levels from adjusting PhaB’s \( K_m \) for (A) acetoacetyl-CoA and (B) NADPH.
The enzyme PHB synthase (PhaC) has a $K_m$ value for the substrate 3-hydroxybutyryl-CoA. Adjusting the $K_m$ value resulted in no visible change in the production of PHB. Figure 122 shows the lack of change in PHB production when compared to the original simulation. Out of the three PHB synthesis enzymes, adjusting $\beta$-ketothiolase resulted in the largest increase in total PHB production.

![Figure 121. PHB production levels from adjusting PhaB’s $K_m$ for acetoacetyl-CoA and NADPH.](image1)

![Figure 122. PHB production levels from adjusting PhaC’s $K_m$ for 3-hydroxybutyryl-CoA.](image2)
**TCA Cycle Enzymes:**

Citrate synthase has a \( K_m \) value for oxaloacetate and acetyl-CoA. Increasing the \( K_m \) for oxaloacetate by five fold resulted in 30.3% increase in PHB production as seen in Figure 123. Multiplying the \( K_m \) for acetyl-CoA by five resulted in an 87.7% increase in PHB production as seen in Figure 123. A large 113.7% increase in PHB production was observed by increasing both \( K_m \) values by five times their original values as seen in Figure 124.

Figure 123. PHB production levels from adjusting citrate synthase’s \( K_m \) for (A) oxaloacetate and (B) acetyl-CoA.

Figure 124. PHB production levels from adjusting citrate synthase’s \( K_m \) for oxaloacetate and acetyl-CoA.
Adjusting the $K_m$ value for citrate hydro-lyase resulted in no visible increase in the production of PHB. Increasing the $K_m$ value for the enzyme isocitrate hydro-lyase resulted in a very slight increase in PHB production. The simulations for these two enzymes can be found in Appendix C.

Adjusting the substrate affinity for isocitrate dehydrogenase yielded a visible change in the amount of PHB synthesized. Decreasing the enzyme’s $K_m$ for isocitrate by one fifth resulted in a 7.8% increase in PHB production as shown in Figure 125. Altering the $K_m$ for the enzyme 2-oxoglutarate dehydrogenase had no visible effect upon the synthesis of PHB. The simulation from adjusting the $K_m$ of 2-oxoglutarate dehydrogenase is found in Appendix C.

![Image](image.png)

Figure 125. PHB production levels from adjusting isocitrate dehydrogenase’s $K_m$ for isocitrate.

The enzyme succinyl-CoA synthetase catalyzed the only reaction not modeled using a kinetic mechanism. The reaction rate was adjusted in place of a $K_m$ value. Increasing the rate of reaction for succinyl-CoA synthetase resulted in a barely noticeable increase in PHB production. This simulation is included in Appendix C.
Adjusting the $K_m$ values for the enzymes succinate dehydrogenase, fumarase, and malate dehydrogenase resulted in no visible increase in PHB production. The simulations for these enzymes are included in Appendix C.

**Glyoxylate Bypass Enzymes:**

Increasing the $K_m$ for the enzyme isocitrate lyase resulted in an 8.6% increase in the synthesis of PHB as shown in Figure 126. For the enzyme malate synthase, no change in PHB production was observed from adjusting the $K_m$ for glyoxylate and a very slight increase in PHB production was observed from increasing the $K_m$ for acetyl-CoA. The two simulations for malate synthase are included in Appendix C.

![Figure 126. PHB production levels from adjusting isocitrate lyase’s $K_m$ for isocitrate.](image)

**Glycolysis Enzymes:**

Altering the $K_m$ values for the enzymes kinase, phosphoglucone isomerase, and 6-phosphofructokinase resulted in no visible increase in the production of PHB. The simulations for these enzymes are found in Appendix C. Increasing the $K_m$ by five fold for the enzyme fructose-bisphosphate aldolase resulted in a 22.5% increase in PHB.
production as seen in Figure 127. For the enzyme triose phosphate isomerase, multiplying the $K_m$ value by five resulted in a 7.1% increase in PHB synthesis as seen in Figure 128.

![Figure 127. PHB production levels from adjusting fructose-bisphosphate aldolase’s $K_m$ for fructose-1,6-bisphosphate.](image1)

![Figure 128. PHB production levels from adjusting triose phosphate isomerase’s $K_m$ for dihydroxyacetone phosphate.](image2)

The enzyme glyceraldehyde-3-phosphate dehydrogenase binds three substrates so it has $K_m$ values for glyceraldehyde-3-phosphate, $\text{NAD}^+$, and inorganic phosphate. Adjusting the $K_m$ for glyceraldehyde-3-phosphate resulted in no visible increase in PHB production and the simulation is included in Appendix C. An 8.6% increase in PHB
synthesis was observed from increasing $K_m$ by five-fold for inorganic phosphate as shown in Figure 129. Increasing the $K_m$ for NAD$^+$ by five-fold resulted in a 37.6% increase in the synthesis of PHB as seen in Figure 129. A total increase of 72.8% in PHB production was observed by increasing all three $K_m$ values by five fold as shown in Figure 130.

Figure 129. PHB production levels from adjusting glyceraldehyde-3-phosphate dehydrogenase’s $K_m$ for (A) NAD$^+$ and (B) inorganic phosphate.

Figure 130. PHB production levels from adjusting glyceraldehyde-3-phosphate dehydrogenase’s $K_m$ for NAD$^+$, glyceraldehyde-3-phosphate, and inorganic phosphate.
No visible increase in PHB production was observed by altering the $K_m$ values for the enzymes phosphoglycerate kinase, phosphoglycerate mutase, and enolase. The enzyme pyruvate kinase has a $K_m$ for phosphoenolpyruvate and ADP. Increasing or decreasing the $K_m$ for phosphoenolpyruvate resulted in no visible change in the production of PHB. A very slight increase in PHB production was observed by increasing the $K_m$ for ADP. The simulations for these four enzymes are available in Appendix C.

*Acetyl-CoA Synthesis Enzymes:*

The only enzyme in the acetyl-CoA synthesis pathway is the multienzyme complex pyruvate dehydrogenase. Figure 131 shows that an increase in PHB production is the result of decreasing the $K_m$ value for the substrate pyruvate. Decreasing $K_m$ by one-fifth resulted in a 26.0% increase in the synthesis of PHB.

![Figure 131. PHB production levels from adjusting pyruvate dehydrogenase’s $K_m$ for pyruvate.](image)

*Enzyme Summary:*

Large increases in PHB production were observed by adjusting the $K_m$ values for $\beta$-ketothiolase, acetoacetyl-CoA reductase, citrate synthase, isocitrate dehydrogenase,
isocitrate lyase, fructose-bisphosphate aldolase, triose phosphate isomerase, glyceraldehyde-3-phosphate dehydrogenase, and pyruvate dehydrogenase. The enzyme acetoacetyl-CoA reductase was the only enzyme where changes in $K_m$ increased the rate of PHB production. The largest percent increase (113.7%) came from adjusting the $K_m$ values for citrate synthase, and the second largest increase (72.8%) came from changing the $K_m$ values for glyceraldehyde-3-phosphate dehydrogenase as seen in Table XLI.

Altering $K_m$ values resulted in a slight increase in PHB production for the enzymes isocitrate hydro-lyase, succinyl-CoA synthetase, malate synthase, phosphoglycerate mutase, and pyruvate kinase. The adjustment of $K_m$ values caused no increase in PHB production for the enzymes PHB synthase, citrate hydro-lyase, 2-oxoglutarate dehydrogenase, succinate dehydrogenase, fumarate, malate dehydrogenase, kinase, phosphoglucose isomerase, 6-phosphofructokinase, phosphoglycerate kinase, enolase, glucose-1-phosphatase, fructose-1,6-bisphosphatase, phosphoenolpyruvate carboxykinase, and phosphoenolpyruvate synthetase.

Table XLI. $K_m$ adjustments leading to increased PHB production. Percent increase is from increasing or decreasing $K_m$ by five-fold.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>$K_m$ A</th>
<th>$K_m$ B</th>
<th>$K_m$ C</th>
<th>% Increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-ketothiolase</td>
<td>↓</td>
<td>NA</td>
<td>NA</td>
<td>35.3</td>
</tr>
<tr>
<td>Acetoacetyl-CoA reductase</td>
<td>↓</td>
<td>↓</td>
<td>NA</td>
<td>8.6*</td>
</tr>
<tr>
<td>Citrate synthase</td>
<td>↑</td>
<td>↑</td>
<td>NA</td>
<td>113.7</td>
</tr>
<tr>
<td>Isocitrate dehydrogenase</td>
<td>↓</td>
<td>NA</td>
<td>NA</td>
<td>7.8</td>
</tr>
<tr>
<td>Isocitrate lyase</td>
<td>↑</td>
<td>NA</td>
<td>NA</td>
<td>8.6</td>
</tr>
<tr>
<td>Fructose-bisphosphate aldolase</td>
<td>↑</td>
<td>NA</td>
<td>NA</td>
<td>22.5</td>
</tr>
<tr>
<td>Triose phosphate isomerase</td>
<td>↑</td>
<td>NA</td>
<td>NA</td>
<td>7.1</td>
</tr>
<tr>
<td>Glyceraldehyde-3-phosphate dehydrogenase</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>72.8</td>
</tr>
<tr>
<td>Pyruvate dehydrogenase</td>
<td>↓</td>
<td>NA</td>
<td>NA</td>
<td>26.0</td>
</tr>
</tbody>
</table>

*Increase in rate
Adjusting Ligand Concentration:

The only ligand adjusted for optimization of PHB production was IPTG. The ligand IPTG binds to the LacI repressor and prevents it from repressing transcription of the PHB synthesis genes. Concentrations of other ligands and transcription factors were not tested for optimization because they are widely used in metabolic pathways so the model cannot account for all of the effects that such changes would have on the cell. No significant increase in PHB production was observed from increasing or decreasing the concentration of IPTG as shown in Figure 132.

![Figure 132. PHB production levels from adjusting concentrations of IPTG.](image)

Adjusting Promoter Activity:

The only promoter tested for model optimization was the lac promoter in the pBHR68 plasmid that regulates transcription of the PHB synthesis genes. It is easier to genetically engineer the plasmid than to genetically engineer promoters in the genome. Also, promoters in the genome often regulate the transcription of multiple genes. This model only accounts for a small number of genes, so it cannot accurately predict the effect of altering a promoter in the genome.
The *lac* promoter that regulates the PHB genes was adjusted to increase PHB production because all the genes in the pBHR68 plasmid are accounted for in the model. Multiplying the *lac* promoter activity (β) by a factor of five resulted in an 8.5% increase in PHB production as seen in Figure 133. Also, maximum PHB production was reached by 12 hours compared to the 43 hours observed at normal promoter activity. A factor of five was used to standardize comparison with the increases observed from adjusting $K_m$ values for the enzymes.

![PHB production levels from adjusting activity (β) of the lac promoter in the pBHR68 plasmid.](image)

**Optimized Model:**

An optimized model for PHB production was created by increasing promoter activity by five fold for the *lac* promoter and by increasing all $K_m$ values by five fold for citrate synthase and glyceraldehyde-3-phosphate dehydrogenase. This optimized model resulted in a 226.8% increase in PHB production achieved by 29 hours compared to 43 hours. Figure 134 shows the comparison between the optimized model and the normal model. Altering the enzymes contributed mostly to the increase in total PHB production,
while altering the promoter activity contributed mostly to the increase in the rate of PHB production as illustrated by Figure 134.

Figure 134. Simulations showing the comparisons between PHB production levels from the unaltered model; PHB production levels from increasing $K_m$ values for citrate synthase and glyceraldehyde-3-phosphate dehydrogenase; PHB production levels from increasing the $pBHR68$ lac promoter activity ($\beta$); and PHB production levels from increasing the $K_m$ values of the two enzymes and increasing the $pBHR68$ lac promoter activity ($\beta$).
DISCUSSION AND FUTURE WORK

Discussion

The predictive model identified nine enzymes as potential targets for genetic engineering in order to optimize the production of PHB. Four of the enzymes required decreasing $K_m$ in order to increase PHB production. Lowering $K_m$ values is equivalent to increasing an enzyme’s substrate affinity. Engineering an enzyme to have a higher substrate affinity is a difficult task because enzymes have evolved to be efficient at binding their substrates. It is easier to make an enzyme less effective at binding its substrate. Increasing the $K_m$ values of five enzymes led to an increase in PHB production. The active sites of these five enzymes could be engineered to be less effective at binding their substrates. Therefore, the nine potential targets for genetic engineering was narrowed down to five enzymes.

Out of the five enzymes, altering the $K_m$ for citrate synthase resulted in the largest increase in PHB production. Due to the $113.7\%$ increase observed by increasing $K_m$ by five fold, citrate synthase is the first target gene for engineering. The two active sites in citrate synthase could be genetically engineered to be less effective at binding the substrates oxaloacetate and acetyl-CoA, and the result should enhance PHB production.

Out of the five enzymes identified as potential targets, increasing the $K_m$ of glyceraldehyde-3-phosphate dehydrogenase caused the second largest increase observed in PHB production. Glyceraldehyde-3-phosphate dehydrogenase is the second target gene for engineering because of the $72.8\%$ increase in PHB synthesis that resulted from increasing the $K_m$ values by five fold. The three active sites for glyceraldehyde-3-
phosphate, NAD\(^+\), and inorganic phosphate could be genetically engineered to be less effective at binding the substrates, and the result should increase PHB production.

The *lac* promoter in the pBHR68 plasmid was the third target identified for genetic engineering. Increasing the *lac* promoter activity by five times resulted in an 8.5% increase in total PHB production and a 275% increase in the rate of PHB production. Genetically engineering the *lac* promoter in the pBHR68 plasmid to have greater activity should result in a significant increase in the rate of PHB production. The only other adjustment that resulted in an increase in the rate of PHB production was decreasing the \(K_m\) by a fifth for the enzyme acetoacetyl-CoA reductase. However, this adjustment only resulted in an 8.6% increase in the rate of PHB production. Therefore, the *lac* promoter in the pBHR68 plasmid was chosen as the third target for genetic engineering.

The simulation that made adjustments for all three targets resulted in a 226.8% increase in maximum PHB production and a 275% increase in the rate of PHB production. Genetically engineering a strain of *E. coli* that can produce more PHB in a shorter amount of time will help make commercial PHB production more cost effective. An optimized strain should be achieved by altering the genes for citrate synthase (*gltA*) and glyceraldehyde-3-phosphate dehydrogenase (*gapA*), and by modifying the *lac* promoter in the pBHR68 plasmid.

Instead of genetically engineering citrate synthase and glyceraldehyde-3-phosphate dehydrogenase to be less effective, substrate analogs could be used as competitive inhibitors for the enzymes. For citrate synthase, the compound carboxymethyl-CoA acts as an inhibitor of the transition state with a high binding
affinity. Theoretically, carboxymethyl-CoA could be added to the bioreactor to inhibit citrate synthase. For glyceraldehyde-3-phosphate dehydrogenase, adenosine analogs can be used as tight-binding inhibitors of the NAD$^+$ active site. Adding an adenosine analog to the bioreactor should slow down the enzyme glyceraldehyde-3-phosphate dehydrogenase.

One major disadvantage of adding a compound to the bioreactor is that the compound could affect more than just the target enzyme. Carboxymethyl-CoA could inhibit other reactions that use acetyl-CoA. Adenosine analogs could inhibit other reactions that use NAD$^+$. Adding compounds to the bioreactor also increases production cost. Due to the cost and potential side effects of adding substrate analogs to the reactor, it was decided to focus first on genetically engineering the enzymes to be less effective.

Flux balance analysis (FBA) has widely been used in genome-scale metabolic models. However, a model based on FBA would not have been able to predict that lowering the substrate affinity of citrate synthase or glyceraldehyde-3-phosphate dehydrogenase would increase PHB production. FBA is limited to predicting how the addition or deletion of gene affects a product’s flux. Deletion of citrate synthase or glyceraldehyde-3-phosphate dehydrogenase would be undesirable because they are both part of key energy pathways in the cell. Also, FBA would not have been able to quantitatively predict how adjusting promoter activity would impact PHB production because FBA does not account for a dynamic transcriptional network.

Certain strains of PHB producing bacteria have been genetically engineered to secrete the produced PHB. Secreting PHB into the growth media can significantly reduce the cost of downstream processing. The secretion pathway could be added to the model to
predict how to optimize a PHB secreting strain of bacteria for greater PHB production. Another way to reduce cost of PHB production is to use a waste material as the initial substrate. Whey is a waste product from cheese production that contains lactose. Lactose could be used instead of glucose in the growth medium. The model could be adjusted to account for the catabolism of lactose instead of glucose.

The design process used to make a predictive model for PHB production can be used to develop predictive models for other bioproducts. Useful bioproducts such as biofuels, biomaterials, and biopharmaceuticals are continually being developed, but often many years of research are required before commercial production becomes cost-effective. Predictive models can be used to develop optimized strains of bacteria for production of specific bioproducts. Predictive models should help reduce time and cost needed to get useful bioproducts out on the market.

Future Work

Metabolic profiling can be used to more precisely fit a predictive model to a specific strain of *E. coli*. Due to the lack of a functioning mass spectrometer, this predictive model was not fitted to metabolic profiling data. This model is not as accurate as it could be if it was fitted to real time data from the specific strain of *E. coli*. Further work should be done to obtain metabolic profiling data to use in the model.

After the model has been fitted to metabolic profiling data, the model should be optimized to verify that altering the effectiveness of citrate synthase, glyceraldehyde-3-phosphate dehydrogenase, and the *lac* promoter in the pBHR68 plasmid will optimize PHB production.
After the target genes have been verified, they should be genetically engineered to mimic the optimized model. The amino acids in the active sites of citrate synthase and glyceraldehyde-3-phosphate dehydrogenase should be identified. One or more of the amino acids should be exchanged with a less effective amino acid through site-directed mutagenesis. This genetic engineering should result in enzymes that are less effective at binding their substrates. The lac promoter in the pBHR68 plasmid should be altered to more closely match the consensus sequence that binds RNA polymerase. This genetic engineering should stabilize the RNA binding and increase the activity of the lac promoter in the pBHR68 plasmid.

Once the strain of *E. coli* has been genetically engineered for optimized PHB production, metabolic profiling should be conducted again. The predictive model should then be fit to the new metabolic profiling data. By comparing the old model with the new data, the accuracy of the first predictive model can be determined. The new predictive model can then be optimized to identify new targets for genetic engineering. This iterative process should be repeated until a fully optimized strain of *E. coli* for PHB production has been genetically engineered.

The PHB predictive model designed in this project is limited to five metabolic pathways and accounts for only a portion of the transcriptional network. These limitations only allow the PHB predictive model to predict outcomes from modifying 30 genes, one promoter, and one ligand. However, in the future the data needed to build a genome-scale kinetic model will become available (Jamshidi & Palsson, 2008). A genome-scale kinetic model would account for all the metabolic pathways and would include a full, dynamic transcriptional network. Such a model would be able to predict
the outcomes of modifying any gene, promoter, transcription factor, or ligand. A genome-scale kinetic model would be a valuable tool in genetically engineering optimized organisms for the production of bioproducts.

The PHB predictive model developed in this project is a step towards one day realizing a genome-scale kinetic model as shown in Table XLII. Although the data needed to build a genome-scale kinetic model is not yet available, progress comes from implementing ideas with the tools that exist today. Engineers need to build working models with the data and technology currently available, and then the models can be improved as new data and technologies are discovered.

Table XLII. Comparisons between the current PHB predictive model and a future genome-scale kinetic model.

<table>
<thead>
<tr>
<th></th>
<th>PHB Predictive Model</th>
<th>Genome-Scale Model</th>
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</thead>
<tbody>
<tr>
<td>Metabolic Pathways:</td>
<td>5</td>
<td>All</td>
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<tr>
<td>Transcriptional Network:</td>
<td>Partial, dynamic</td>
<td>Full, dynamic</td>
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<tr>
<td>Predict Outcomes from Modifying:</td>
<td>30 genes</td>
<td>Any gene</td>
</tr>
<tr>
<td></td>
<td>1 promoter</td>
<td>Any promoter</td>
</tr>
<tr>
<td></td>
<td>1 ligand</td>
<td>Any ligand</td>
</tr>
<tr>
<td></td>
<td>0 transcription factors</td>
<td>Any transcription factor</td>
</tr>
</tbody>
</table>
REFERENCES


Anderson DH, Duckworth HW (1988) In vitro mutagenesis of Escherichia coli citrate synthase to clarify the locations of ligand binding sites. Biol Chem 263: 2163-2169


Doi Y (1990) *Microbial polyesters*. Wiley-VCH, New York City, NY, USA


Mills SA, Marletta MA (2005) Metal binding characteristics and role of iron oxidation in the ferric uptake regulator from Escherichia coli. Biochemistry 44: 13553-13559


Perdiguero E, Arriaga D, Busot F, Soler J (1995) Isocitrate lyase from *Cephalosporium acremonium*. Role of Mg$^{2+}$ ions, kinetics, and evidence for a histidine residue in the active site of the enzyme. Biochemistry 34: 6059-6068


Wang X, Kemp RG (1999) Identification of residues of *Escherichia coli* phosphofructokinase that contribute to nucleotide binding and specificity. *Biochemistry* **38**: 4313-4318


APPENDICES
Appendix A. Transcription Factor Chart
Appendix B. HPLC-MS Analytical Methods
HPLC-MS Analytical Methods

Metabolic profiling samples can be analyzed for concentration of metabolites using high performance liquid chromatography coupled with mass spectrometry (HPLC-MS). Standards should be used to identify and analyze concentrations of glucose, pyruvate, citrate, 2-oxoglutarate, succinate, fumarate, malate, oxaloacetate, and beta-hydroxybutyrate. Dilutions of the standards should be used to create a standard curve for each compound.

Mass spectrometric analysis can be conducted on an Agilent 6130 single quadrupole mass spectrometer. High performance liquid chromatography can be performed on an Agilent 1200 Series HPLC. Solvent A should be an aqueous buffer, and Solvent B should be acetonitrile. Two methods could be used to separate desired metabolites. Method 1 is a reversed-phase liquid chromatography utilizing a C18 column. Method 2 is a hydrophilic interaction liquid chromatography utilizing an aminopropyl column (Bajad et al, 2006).

Concentrations of the metabolites can be analyzed for each time sample utilizing HPLC-MS. An independent two-sample student’s t-statistic test could then be used to compare the differences in concentrations of the above-mentioned metabolites between the strain containing the PHB producing plasmid and the control to determine if the addition of the PHB synthesis pathway significantly alters the kinetics of glycolysis and the TCA cycle.
Appendix C. Simulations
Figure 135. PHB production levels from adjusting (A) citrate hydro-lyase’s $K_m$ for citrate, (B) isocitrate hydro-lyase’s $K_m$ for aconitate, (C) oxoglutarate dehydrogenase’s $K_m$ for 2-oxoglutarate, (D) succinyl-CoA synthetase’s reaction rate, (E) succinate dehydrogenase’s $K_m$ for succinate, and (F) fumarase’s $K_m$ for fumarate.
Figure 136. PHB production levels from adjusting (A) malate dehydrogenase’s $K_m$ for malate, (B) malate dehydrogenase’s $K_m$ for NAD$,^+$ (C) malate synthase’s $K_m$ for glyoxylate, (D) malate synthase’s $K_m$ for acetyl-CoA, (E) kinase’s $K_m$ for glucose, and (F) kinase’s $K_m$ for ATP.
Figure 137. PHB production levels from adjusting (A) phosphoglucone isomerase’s $K_m$ for glucose-6-phosphate, (B) phosphoglucone isomerase’s $K_m$ for fructose-6-phosphate, (C) 6-phosphofructokinase’s $K_m$ for fructose-6-phosphate, (D) 6-phosphofructokinase’s $K_m$ for ATP, (E) glyceraldehyde-3-phosphate dehydrogenase’s $K_m$ for glyceraldehyde-3-phosphate, and (F) phosphoglycerate mutase’s $K_m$ for 3-phosphoglycerate.
Figure 138. PHB production levels from adjusting (A) phosphoglycerate kinase’s $K_m$ for 1, 3-diphosphoglycerate, (B) phosphoglycerate kinase’s $K_m$ for ADP, (C) enolase’s $K_m$ for 2-phosphoglycerate, (D) pyruvate kinase’s $K_m$ for phosphoenolpyruvate, and (E) pyruvate kinase’s $K_m$ for ADP.
Appendix D. SimBiology Model Tutorial
Instructions are for SimBiology Version 3.2 (R2010a)

Download File

Predictive Model: [http://digitalcommons.usu.edu/engineering_datasets/1/](http://digitalcommons.usu.edu/engineering_datasets/1/)

Open SimBiology Project

Open MATLAB

Type “sbiodesktop” to open SimBiology

Go to File, then select Open Project, then select the Predictive Model

How to Open Diagram View:

In the Project Explorer window pane, expand Model Session, then expand SimBiology Model and select Diagram View

![Project Explorer](image)

Editing Properties of Species

Double-click on the Species block

Settings Tab

- Here changes can be made to the initial amount of the species
- Select whether the species is held constant, or if the amount is determined by a boundary condition
Editing Properties of Reactions

Double-click on the Reaction block

Settings

- Here changes can be made to the kinetic law and parameters
- Reaction rate expression can be edited by clicking on the pencil icon
- $K_m$ values can be changed by double-clicking the Value cell for $K_m$ and entering a new value
- Make a reaction unactive during the simulation by unchecking the Active checkbox
Cloned/Split Species

- A block is cloned or split to separate a species that participates in multiple reactions.
- Although there could be multiple split/clone blocks, there is only one species. Changes made to any settings in the **Species Properties** dialog box will be propagated to each of the split/clone blocks.

### Parameter Properties

**Changing a Parameter’s Scope:**

- In the **Project Explorer** window pane select **Parameters**
- **Right-click** on the desired parameter in the **Scope** column. Select “**Change Parameter Scope.**” This toggles the scope between the reaction and the overall model. A parameter must have a global scope if it is used in an **Event** or a **Rule**.

**Constant:**

- The default setting for all parameters is for them to be held constant throughout the entire simulation. To change the parameters value partway through the simulation (through use of an **Event**), then uncheck the **Constant Value** box in the parameter settings.

### Rules

In the **Project Explorer** window pane select **Rules**

#### 4 Types of Rules:

1. Algebraic — Evaluated continuously during a simulation
2. Initial Assignment — Evaluated once at the beginning of the simulation
3. Repeated Assignment — Evaluated at every time-step of the simulation
4. Rate — Evaluated continuously during the simulation

Tip: To use a parameter in a rule, remember to set the Scope of the parameter to “model.” To use an algebraic or rate rule to vary the value of a parameter during the simulation, clear the ConstantValue checkbox for the parameter in the Parameters pane. Also, if using a rule to define a species concentration, clear the ConstantAmount checkbox and check the BoundaryCondition checkbox.

Events

In the Project Explorer window pane select Events

Events are used to describe sudden changes in model behavior. An event can specify discrete transitions in model component values that occur when a user-specified condition become true.

Uses:
- To activate or deactivate certain species (activator or inhibitor species), change parameter values, change reaction rates in response to addition or removal of species

Trigger:
- The trigger can be time-dependent or time-independent

Event Functions:
- Event functions are the results when the event is turned on by the trigger

Active:
- The Active check box must be marked for the event to work. To simulate the model without the event, then clear the Active check box.

Tip:
- To use a parameter in an event, remember to set the Scope of the parameter to model and to clear the ConstantValue check box for the parameter in the Parameters pane. Also, the solver must be set to sundial in order to simulate events.
Configuration Settings

In the Project Explorer window pane expand Model Session and select Configuration Settings

Before running a simulation, use the Configuration Settings to set the simulation time, solver, etc.

Custom Configuration Settings:

To store simulation settings for later use, type a name for the settings in the Enter Name edit box, and click Add to create your custom configuration settings. Any changes made in the Settings and Data Logging tabs will be saved with the project.

Custom Configuration Settings Available in Predictive Model

- CoFactor: Simulates cofactors in model
- PHB synthesis: Simulates species in PHB synthesis pathway
- TCA cycle: Simulates species in TCA and Glyoxylate pathways
- Glycolysis: Simulates species in the Glycolysis pathway
- Glucose_PHB: Simulates key species of the entire model

Settings:

- Set simulation time
- Choose solver (Use sundial if using Events)
- Select the species to be logged

Simulation

In the Project Explorer window pane expand Model Session, then expand Model Tasks and select Simulation

- In the Settings tab, select the Configuration Settings to use for the simulation
- Next, select the Plots tab
  - Double click the “y” argument box to select which species to plot
  - Under Plot Behavior, select if the simulation should be a new figure or if it should be added to the current axes
- Click the Run icon in the main Toolbar
- A graph of your simulation will appear in the Figures window pane
Importing Data

In the main task bar, select File, and then select Add Data, and choose From File

- Select a file to download
- Select if the first row contains header information
- Look at the Data Preview, and if it looks good, then click Okay

Plotting Multiple Simulations in a Single Figure

In the Project Explorer window pane expand Model Session, then expand Model Tasks and select Simulation

- In the Plots tab, select whether the plot should be put in a new Figure or if the plot should be added to the current axes. If “Add to current axes” is selected then the simulation will be plotted on the Figure last selected. This is the best way to overlay different simulations.

Plotting a Dataset with a Simulation in a Single Figure

In the Project Explorer window pane expand Model Session, then expand Model Tasks and select Simulation

- In the Plots tab, select an XY Plot Type and click “Add Plot Type”
- For the first plot (Time Plot Type), choose the Plot Behavior “New Figure”
  - Double-click on the “y” argument to select which species to plot from the simulation.
- For the second plot (XY Plot Type), choose the Plot Behavior “Add to current axes”
  - Double-click on the “x” argument to select the x-variable from the imported dataset. Double-click on the “y” argument to select the y-variable from the imported dataset.
  - As long as both Create Plot checkboxes are checked, a Figure will be created containing the simulation and the dataset