ABE 30100

Microbial Consortium Modeling

Deliverable III

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REVIEW OF DELIVERABLE I

BACKGROUND

CONCEPT IN LITERATURE

Fermentation is a process used to exploit microorganisms' ability to produce natural metabolites to the benefit of humans. Organisms such as *Escherichia coli* and *Saccharomyces cerevisiae* have been engineered to ferment products such as insulin and ethanol for human consumption. However, there is a limit to the ability of single-organism fermentations to produce more complex molecules whose building blocks require compartmentalized production to most efficiently create the final product.

In their 2015 Nature Biotechnology paper, Zhou, Qiao, Edgar, and Stephanopoulos fermented *E. coli* and *S. cerevisiae* together to create paclitaxel, a chemotherapy drug (Figure 1).

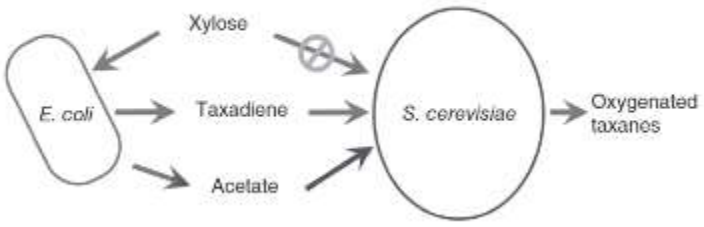

Figure 1: Picture of the fermentation process to be modeled. The E. coli consumes xylose and produces acetate for the S. cerevisiae to uses as a carbon source. E. coli produce taxadiene for the S. cerevisiae to oxygenate and use to produce the final product, paclitaxel (Zhou, Qiao, Edgar, & Stephanopoulos, Distributing a metabolic pathway among a microbial consortium enhances production of natural products, 2015).

The simpler *E. coli* cells were engineered to produce the building blocks of the final product while the *S. cerevisiae* was programmed to fold these building blocks together to produce paclitaxel (Figure 2, Figure 3). The co-culture was fed xylose, a carbon source that only the *E. coli* cells could metabolize to then

produce acetate, a toxin to *E. coli* which *S. cerevisiae* cells could consume for carbon. This, among other genetically engineered tweaks to make the process more streamlined, ensured that neither the *E. coli* nor the *S. cerevisiae* populations overgrew.

Model Proposal

While the authors proved this concept in the lab, a mathematical model of the process was never made, or at least never published. As such, I would like to create a model of the final system that the authors described in their paper, outlined above. My model would output the amount of paclitaxel produced by a certain number of *E. coli* and *S. cerevisiae* cells given an initial amount of xylose in a reactor of specified volume with a defined initial temperature and pH.

MODEL DESCRIPTION

Quantitative Outputs

- Rate of paclitaxel produced [mass/time]

Input Parameters

- Initial temperature
- Initial pH
- Volume of fermenter
- Initial number of *E. coli* cells
- Initial number of *S. cerevisiae* cells
- Initial amount of xylose [mass]

Principles and Processes Modeled

- Conservation of mass
- Conservation of energy
- Mass balance with reaction
- Enzymatic reactions
- Reaction kinetics
- Heat of reaction
- Batch reactor process
- Mass transfer across a membrane
- Diffusion
- Heat transfer
- Cell growth and death

DELIVERABLE II

DEFINING THE MODEL

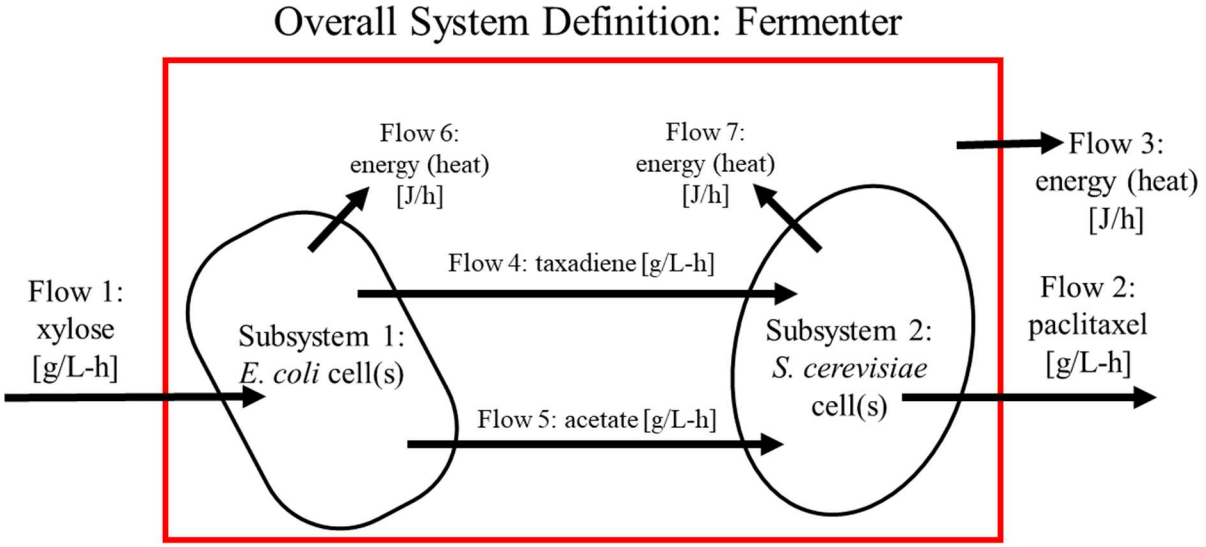

Figure 2: System definition with input and output flows.

MATHEMATICAL EQUATIONS

OVERALL MASS BALANCE

Accumulation = In – Out + Generation – Consumption

- Law of Conservation of Mass: mass can neither be created nor destroyed
  - Generation = Consumption = 0

Accumulation = In – Out

- Figure 2: In = Flow 1; Out = Flow 2

$$\frac{\partial m}{\partial t} = F_1 C_1 - F_2 C_2 \qquad [1]$$

Unit Analysis: $\left[\frac{mass}{time}\right] = \left[\frac{volume}{time} \cdot \frac{mass}{volume}\right] - \left[\frac{volume}{time} \cdot \frac{mass}{volume}\right] = \left[\frac{mass}{time}\right]$

SUBSYSTEM 1 OVERALL MASS BALANCE

Accumulation = In – Out + Generation – Consumption

- Law of Conservation of Mass: mass can neither be created nor destroyed
  - Generation = Consumption = 0

Accumulation = In – Out

- Figure 2: In = Flow 1; Out = Flow 4, Flow 5

$$\frac{\partial m_{s1}}{\partial t} = F_1 C_1 - (r_{x,d} + r_{x,a}) W_x V_{s1} \qquad [2]$$

Unit Analysis: $\left[\frac{mass}{time}\right] = \left[\frac{volume}{time} \cdot \frac{mass}{volume}\right] - \left(\left[\frac{mol}{volume \cdot time}\right] + \left[\frac{mol}{volume \cdot time}\right]\right)\left[\frac{mass}{mol}\right][volume] = \left[\frac{mass}{time}\right]$

SUBSYSTEM 2 OVERALL MASS BALANCE

Accumulation = In – Out + Generation – Consumption

- Law of Conservation of Mass: mass can neither be created nor destroyed
    - Generation = Consumption = 0

Accumulation = In – Out

- Figure 2: In = Flow 4, Flow 5; Out = Flow 2

$$\frac{\partial m_{s2}}{\partial t} = (r_{x,d} + r_{x,a})W_x V_{s1} - F_2 C_2 \quad [3]$$

Unit Analysis: $\left[\frac{mass}{time}\right] = \left(\left[\frac{mol}{volume \cdot time}\right] + \left[\frac{mol}{volume \cdot time}\right]\right)\left[\frac{mass}{mol}\right][volume] - \left[\frac{volume}{time} \cdot \frac{mass}{volume}\right] = \left[\frac{mass}{time}\right]$

Mass Balance on Individual Components

XYLOSE

Accumulation = In – Out + Generation – Consumption

- Figure 2: In = Flow 1; Out = 0
- Assumption #: Generation = 0
- Figures 3 – 5: Consumption = metabolism of xylose to produce taxadiene, acetate, and *E. coli* cell growth

$$\frac{\partial x}{\partial t} = F_1 C_1 - (r_{x,e} + r_{x,d} + r_{x,a})W_x V_{s1} \quad [4]$$

Unit Analysis: $\left[\frac{mass}{time}\right] = \left[\frac{volume}{time} \cdot \frac{mass}{volume}\right] - \left(\left[\frac{mol}{volume \cdot time}\right] + \left[\frac{mol}{volume \cdot time}\right] + \left[\frac{mass}{volume \cdot time}\right]\right)\left[\frac{mass}{mol}\right][volume] = \left[\frac{mass}{time}\right]$

- Note: The consumption of xylose to produce cell growth ($r_{x,e}$) is dependent upon the concentration of xylose, the concentration of acetate (as acetate inhibits *E. coli* cell growth), and the total concentration of cells in the reactor (due to space constraint inhibition). The inhibition considerations will be reflected in future iterations.

PACLITAXEL

Accumulation = In – Out + Generation – Consumption

- Figure 2: In = 0; Out = Flow 2
- Assumption #: Consumption = 0
- Figure 3: Generation = metabolism of taxadiene to produce paclitaxel

$$\frac{\partial p}{\partial t} = r_{d,p} W_d V_{s2} - F_2 C_2 \quad [5]$$

Unit Analysis: $\left[\frac{mass}{time}\right] = \left[\frac{mol}{volume \cdot time}\right]\left[\frac{mass}{mol}\right][volume] - \left[\frac{volume}{time} \cdot \frac{mass}{volume}\right] = \left[\frac{mass}{time}\right]$

TAXADIENE

Accumulation = In – Out + Generation – Consumption

- Figure 2: In = 0; Out = 0
- Figures 3 – 5: Generation = metabolism of xylose to produce taxadiene; Consumption = metabolism of taxadiene to produce paclitaxel

$$\frac{\partial d}{\partial t} = r_{x,d} W_x V_{s1} - r_{d,p} W_d V_{s2} \quad [6]$$

Unit Analysis: $\left[\frac{mass}{time}\right] = \left[\frac{mol}{volume \cdot time}\right]\left[\frac{mass}{mol}\right][volume] - \left[\frac{mol}{volume \cdot time}\right]\left[\frac{mass}{mol}\right][volume] = \left[\frac{mass}{time}\right]$

ACETATE

Accumulation = In – Out + Generation – Consumption

- Figure 2: In = 0; Out = 0
- Figures 5 – 6: Generation = metabolism of xylose to produce acetate; Consumption = metabolism of acetate to produce *S. cerevisiae* cell growth

$$\frac{\partial a}{\partial t} = r_{x,a} W_x V_{s1} - r_{a,s} W_a V_{s2} \quad [7]$$

Unit Analysis: $\left[\frac{mass}{time}\right] = \left[\frac{mol}{volume \cdot time}\right]\left[\frac{mass}{mol}\right][volume] - \left[\frac{mol}{volume \cdot time}\right]\left[\frac{mass}{mol}\right][volume] = \left[\frac{mass}{time}\right]$

E. COLI CELLS

Accumulation = In – Out + Generation – Consumption

- Figure 2: In = 0; Out = 0
- Figure 5: Generation = metabolism of xylose to produce cell growth; Consumption = cell death

$$\frac{\partial e}{\partial t} = r_{x,e} W_x V_{s1} - f(x, a, (e + s), T) \quad [8]$$

Unit Analysis: $\left[\frac{mass}{time}\right] = \left[\frac{mol}{volume \cdot time}\right]\left[\frac{mass}{mol}\right][volume] - \left[\frac{mass}{time}\right] = \left[\frac{mass}{time}\right]$

- Note that cell death is defined as a function of the concentrations of xylose, acetate, and total cell mass and temperature. This function will be fleshed out in future iterations where cell death is assumed to be nonzero.

S. CEREVISIAE CELLS

Accumulation = In – Out + Generation – Consumption

- Figure 2: In = 0; Out = 0
- Figures 6: Generation = metabolism of acetate to produce cell growth; Consumption = cell death

$$\frac{\partial s}{\partial t} = r_{a,s} W_a V_{s2} - f(a, (e + s), T) \quad [9]$$

Unit Analysis: $\left[\frac{mass}{time}\right] = \left[\frac{mol}{volume \cdot time}\right]\left[\frac{mass}{mol}\right][volume] - \left[\frac{mass}{time}\right] = \left[\frac{mass}{time}\right]$

- Note that cell death is defined as a function of the concentration of acetate and total cell mass and temperature. This function will be fleshed out in future iterations where cell death is assumed to be nonzero.

OVERALL ENERGY BALANCE

Accumulation = In – Out + Generation – Consumption

- Figure 2: In = 0; Out = Flow 3; Generation = Flow 6, Flow 7

$$\frac{\partial E}{\partial t} = F_6 + F_7 - F_3 \quad [10]$$

Unit Analysis: $\left[\frac{energy}{time}\right] = \left[\frac{energy}{time}\right] + \left[\frac{energy}{time}\right] - \left[\frac{energy}{time}\right] = \left[\frac{energy}{time}\right]$

SUBSYSTEM 1 ENERGY BALANCE
Accumulation = In – Out + Generation – Consumption

- Figure 2: In = 0; Out = Flow 6
- Figures 3 – 5: [Generation – Consumption] = lumped heats of reactions of metabolism of xylose to produce taxadiene, acetate, and *E. coli* cell growth

$$\frac{\partial E_{s1}}{\partial t} = \left(H_{x,d}r_{x,d} + H_{x,a}r_{x,a} + H_{x,e}r_{x,e}\right)W_x V_{s1} - F_6 \qquad [11]$$

Unit Analysis: $\left[\frac{energy}{time}\right] = \left(\left[\frac{energy}{mole}\right]\left[\frac{mole}{mass}\right]\left[\frac{mass}{volume \cdot time}\right][volume] + \left[\frac{energy}{mole}\right]\left[\frac{mole}{mass}\right]\left[\frac{mass}{volume \cdot time}\right][volume] + \left[\frac{energy}{mole}\right]\left[\frac{mole}{mass}\right]\left[\frac{mass}{volume \cdot time}\right][volume]\right) - \left[\frac{energy}{time}\right] = \left[\frac{energy}{time}\right]$

SUBSYSTEM 2 ENERGY BALANCE
Accumulation = In – Out + Generation – Consumption

- Figure 2: In = 0; Out = Flow 7
- Figures 3, 6: [Generation – Consumption] = lumped heats of reactions of metabolism of taxadiene and acetate to produce paclitaxel and *S. cerevisiae* cell growth

$$\frac{\partial E_{s2}}{\partial t} = \left(H_{d,p}W_d r_{d,p} + H_{a,s}W_a r_{a,s}\right)V_{s2} - F_7 \qquad [12]$$

Unit Analysis: $\left[\frac{energy}{time}\right] = \left(\left[\frac{energy}{mole}\right]\left[\frac{mole}{mass}\right]\left[\frac{mass}{volume \cdot time}\right][volume] + \left[\frac{energy}{mole}\right]\left[\frac{mole}{mass}\right]\left[\frac{mass}{volume \cdot time}\right][volume]\right) - \left[\frac{energy}{time}\right] = \left[\frac{energy}{time}\right]$

RELEVANT PARAMETERS, RELATIONSHIPS, AND PRINCIPLES

PARAMETERS

- See Appendix A for parameter nomenclature and descriptions

RELATIONSHIPS

- $r = kC_{reactant}^{order}$
  - This is used to determine the reaction rates based on the concentration(s) of the reactant(s)
- $\frac{dE}{dt} = UA\frac{dT}{dt}$
  - This is used for determining the temperature of the cells, of the broth, and of the water used to cool the broth based on the energy produced by the cellular reactions
- $V = \frac{m}{\rho}$
  - This is used for determining the volume of cells based upon their concentration in the reactor

PRINCIPLES

- Conservation of mass
- Conservation of energy
- Reaction kinetics
- Heat transfer

ASSUMPTIONS

1. The fermentation broth is perfectly homogeneous in composition and temperature
2. The fermentation broth is kept at a constant temperature
3. The fermentation broth is kept at a constant volume
4. The fermentation broth is kept at a constant pH
5. All *E. coli* cells are identical to each other and all *S. cerevisiae* cells are identical to each other
6. Cells neither grow nor die
7. Each reaction is zeroth order
8. Transportation across the cell membrane is instantaneous and requires no energy
9. The cells have enough enzymes and cellular resources to perform each reaction
10. There is no wait time between reactions (i.e. no need for reaction products to diffuse within the cell or between cells before the next reaction occurs)
11. The output flow is filtered and does not remove any cells, only the desired product and water
12. The mass of water that enters the reactor is equal to the mass of water that leaves the reactor
13. The normal metabolisms of the *E. coli* and *S. cerevisiae* cells do not produce any additional heat
14. All reactions occur to completion
15. If there is more than one pathway to a desired product, only the most direct pathway is taken (i.e. the pathway with the least number of reactions)
16. Reactions only occur in the forward direction
17. Reaction rates are the same at all temperatures
18. If one reactant is used in multiple reactions, the mass is split evenly between each of the reactions

DELIVERABLE III

ITERATION I

ASSUMPTIONS

1. The fermentation broth is perfectly homogeneous in composition and temperature
2. The fermentation broth is kept at a constant temperature
3. The fermentation broth is kept at a constant volume
4. The fermentation broth is kept at a constant pH
5. All *E. coli* cells are identical to each other and all *S. cerevisiae* cells are identical to each other
6. Cells neither grow nor die
7. Each reaction is zeroth order
8. Transportation across the cell membrane is instantaneous and requires no energy
9. The cells have enough enzymes and cellular resources to perform each reaction
10. There is no wait time between reactions (i.e. no need for reaction products to diffuse within the cell or between cells before the next reaction occurs)
11. The output flow is filtered and does not remove any cells, only the desired product and water
12. The mass of water that enters the reactor is equal to the mass of water that leaves the reactor
13. The normal metabolisms of the *E. coli* and *S. cerevisiae* cells do not produce any additional heat
14. All reactions occur to completion
15. If there is more than one pathway to a desired product, only the most direct pathway is taken (i.e. the pathway with the least number of reactions)
16. Reactions only occur in the forward direction
17. Reaction rates are the same at all temperatures

MATHEMATICAL MODEL

See Appendix C for the code used to produce the below output.

Values for reaction rates come from references found in BRENDA: (Agranoff, Eggerer, Henning, & Lynen, 1960), (Bloch, Chaykin, Phillips, & De Waard, 1959), (Cane, Chow, Lillo, & Kang, 2001), (Chau, Walker, Long, & Croteau, 2004), (Chesters, Wilding, Goodall, & Micklefield, 2012), (Durr & Rudney, 1960), (Fang & Ewald, 2004), (Feigenbaum & Schulz, 1975), (Gogerty & Bobik, 2010), (Hahn, et al., 2001), (Inui, Miyatake, Nakano, & Kitaoka, 1990), (Jennewein, Long, Williams, & Croteau, 2004), (Lee, Cheong, & Kim, 2008), (Malcovati & Valentini, 1982), (Mercade, Cocaign-Bousquet, & Loubiere, 2006), (Middleton, 1972), (Nawarathne & Walker, 2010), (Takenoya, et al., 2010), (Voronovsky, et al., 2005), (Walker, Fujisaki, Long, & Croteau, 2002), (Wolff, et al., 2003), and (Yu, Ladapo, & Whitman, 1994).

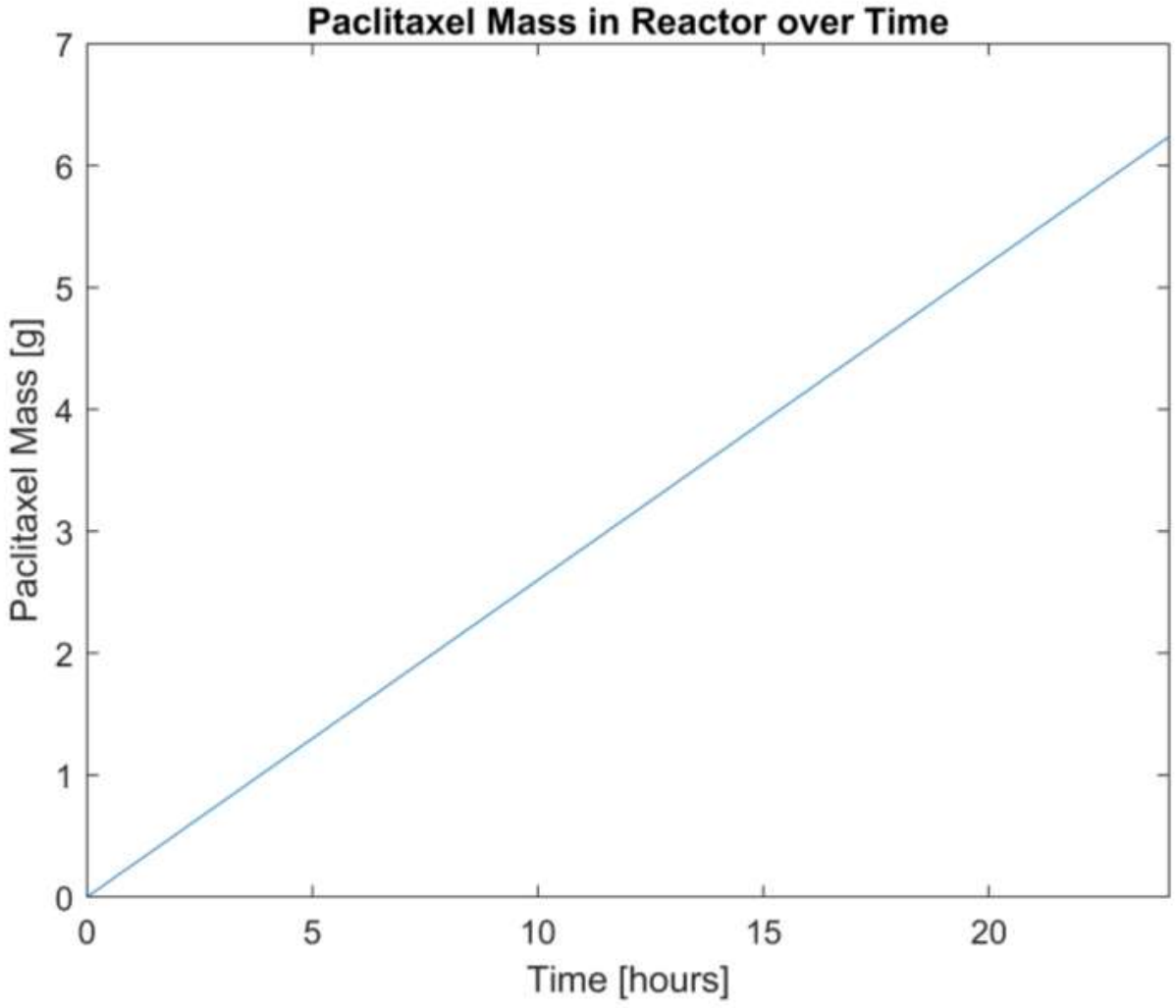

Figure 3: Graphical output of Microbial Consortium Model Iteration I

MODEL EVALUATION

The model is very inaccurate. It shows a linear relationship between the output of Paclitaxel mass and the reaction time with no consideration of a limit on the mass of Paclitaxel that can be produced from the

initial xylose mass (5 g). The next iteration will impose limits on the mass of Paclitaxel that can be produced from the xylose.

ITERATION II

ASSUMPTIONS

1. The fermentation broth is perfectly homogeneous in composition and temperature
2. The fermentation broth is kept at a constant temperature
3. The fermentation broth is kept at a constant volume
4. The fermentation broth is kept at a constant pH
5. All *E. coli* cells are identical to each other and all *S. cerevisiae* cells are identical to each other
6. Cells neither grow nor die
7. Each reaction is zeroth order
8. Transportation across the cell membrane is instantaneous and requires no energy
9. The cells have enough enzymes and cellular resources to perform each reaction
10. There is no wait time between reactions (i.e. no need for reaction products to diffuse within the cell or between cells before the next reaction occurs)
11. The output flow is filtered and does not remove any cells, only the desired product and water
12. The mass of water that enters the reactor is equal to the mass of water that leaves the reactor
13. The normal metabolisms of the *E. coli* and *S. cerevisiae* cells do not produce any additional heat
14. All reactions occur to completion
15. If there is more than one pathway to a desired product, only the most direct pathway is taken (i.e. the pathway with the least number of reactions)
16. Reactions only occur in the forward direction
17. Reaction rates are the same at all temperatures

MATHEMATICAL MODEL

See Appendix C for the code used to produce the below output.

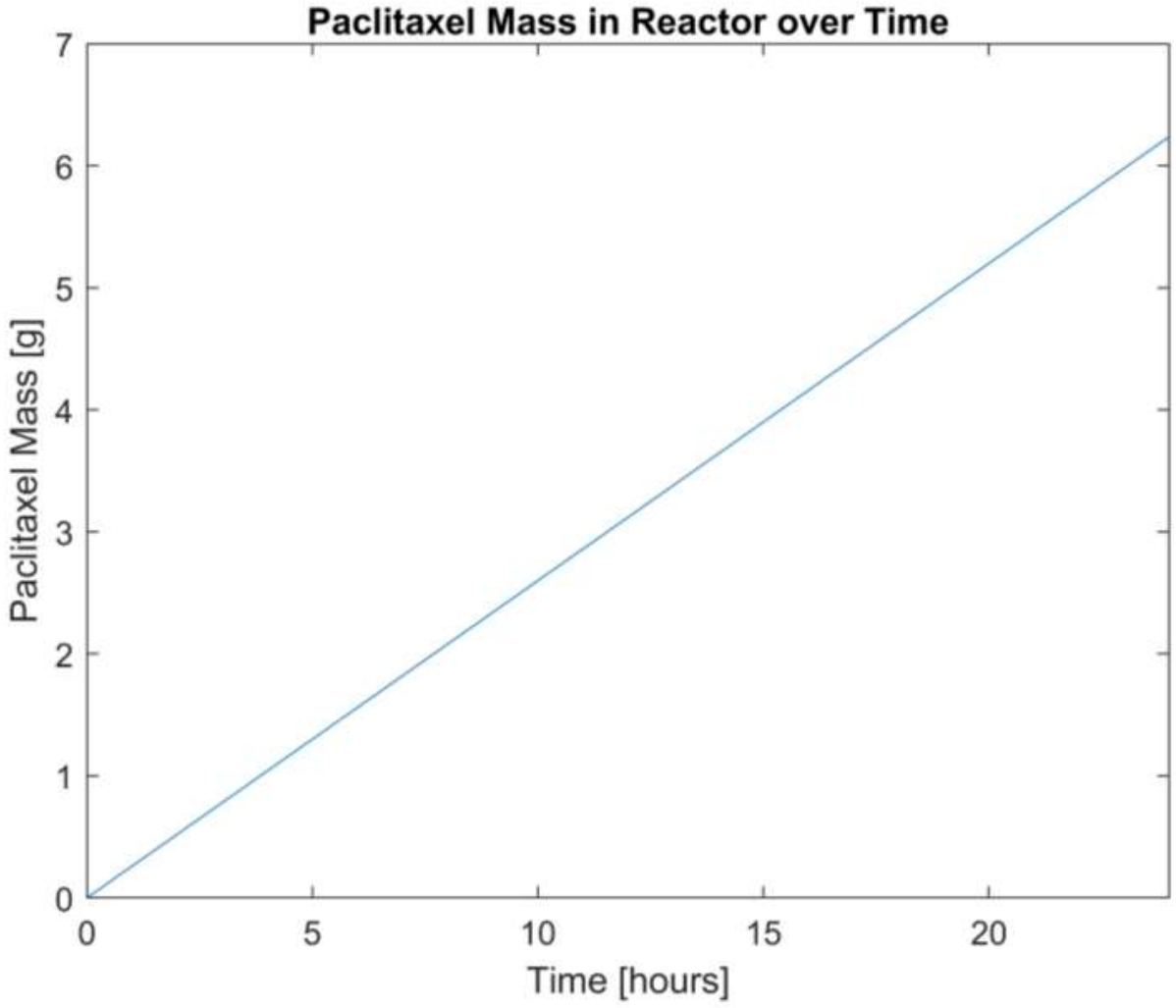

Figure 4: Graphical output of Microbial Consortium Model Iteration II

MODEL EVALUATION

The model still shows a linear relationship between the output of Paclitaxel mass and the reaction time. However, the rate of reaction (slope of the output line) should not remain constant as the resources decrease. The next iteration will change the assumption that the reactions are all zeroth order.

ITERATION III

ASSUMPTIONS

1. The fermentation broth is perfectly homogeneous in composition and temperature
2. The fermentation broth is kept at a constant temperature
3. The fermentation broth is kept at a constant volume
4. The fermentation broth is kept at a constant pH
5. All *E. coli* cells are identical to each other and all *S. cerevisiae* cells are identical to each other
6. Cells neither grow nor die
7. **Each reaction is first order**
8. Transportation across the cell membrane is instantaneous and requires no energy

9. The cells have enough enzymes and cellular resources to perform each reaction
10. There is no wait time between reactions (i.e. no need for reaction products to diffuse within the cell or between cells before the next reaction occurs)
11. The output flow is filtered and does not remove any cells, only the desired product and water
12. The mass of water that enters the reactor is equal to the mass of water that leaves the reactor
13. The normal metabolisms of the *E. coli* and *S. cerevisiae* cells do not produce any additional heat
14. All reactions occur to completion
15. If there is more than one pathway to a desired product, only the most direct pathway is taken (i.e. the pathway with the least number of reactions)
16. Reactions only occur in the forward direction
17. Reaction rates are the same at all temperatures

Mathematical Model

See Appendix C for the code used to produce the below output.

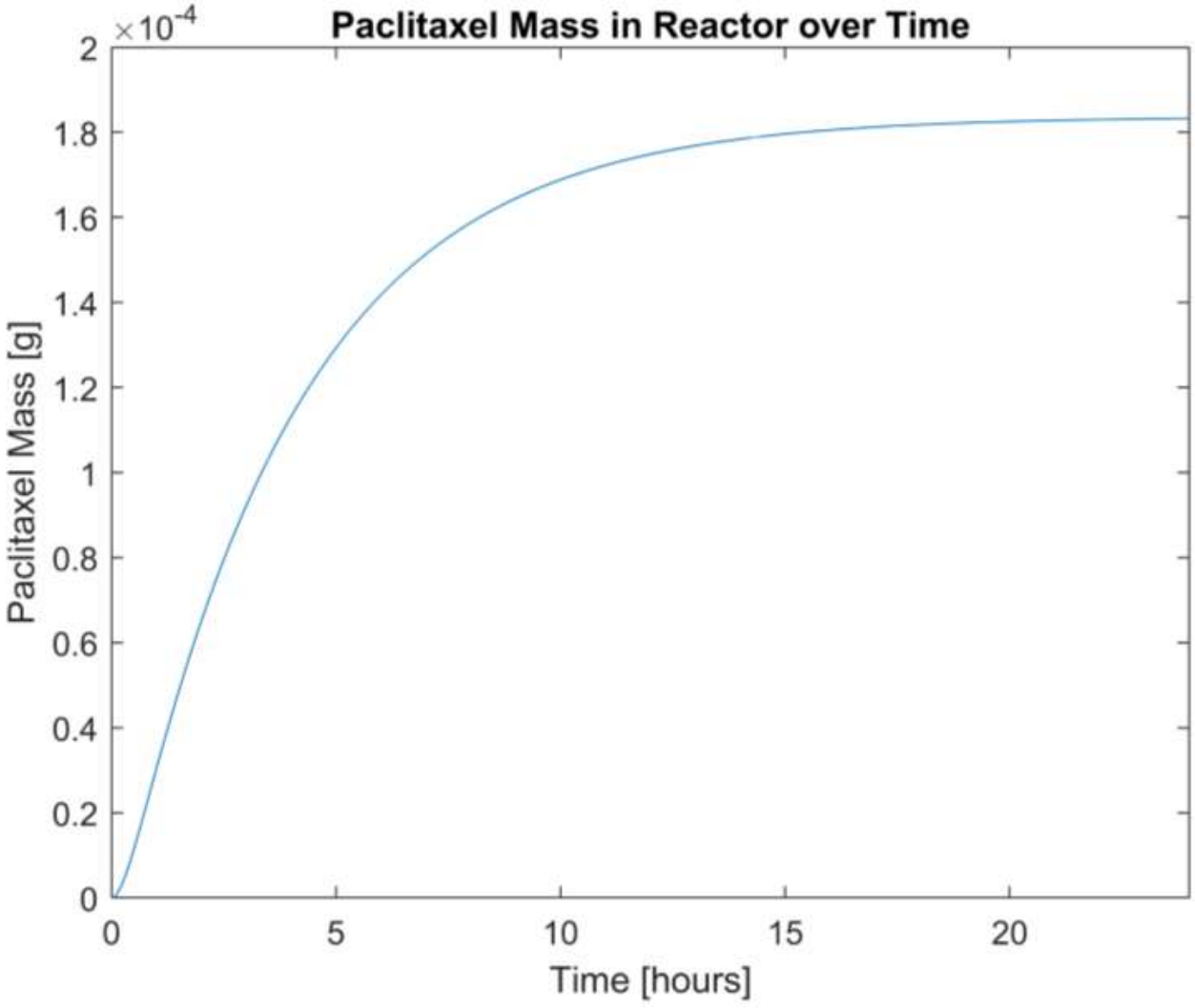

Figure 5: Graphical output of Microbial Consortium Model Iteration III

Model Evaluation

The shape of the curve is more realistic and closer to what I had expected the output of the model to be. However, the model assumes that the rates of reaction in the *E. coli* cells are determined by the entire concentration of xylose even though all the xylose is not used by both reactions. The next iteration will add an assumption to correct this.

ITERATION IV

Assumptions

1. The fermentation broth is perfectly homogeneous in composition and temperature
2. The fermentation broth is kept at a constant temperature
3. The fermentation broth is kept at a constant volume
4. The fermentation broth is kept at a constant pH
5. All *E. coli* cells are identical to each other and all *S. cerevisiae* cells are identical to each other
6. Cells neither grow nor die
7. Each reaction is first order
8. Transportation across the cell membrane is instantaneous and requires no energy
9. The cells have enough enzymes and cellular resources to perform each reaction
10. There is no wait time between reactions (i.e. no need for reaction products to diffuse within the cell or between cells before the next reaction occurs)
11. The output flow is filtered and does not remove any cells, only the desired product and water
12. The mass of water that enters the reactor is equal to the mass of water that leaves the reactor
13. The normal metabolisms of the *E. coli* and *S. cerevisiae* cells do not produce any additional heat
14. All reactions occur to completion
15. If there is more than one pathway to a desired product, only the most direct pathway is taken (i.e. the pathway with the least number of reactions)
16. Reactions only occur in the forward direction
17. Reaction rates are the same at all temperatures
18. **If a reactant is used in more than one reaction, the mass of the reactant will be split evenly between the reactions.**

Mathematical Model

See Appendix C for the code used to produce the below output.

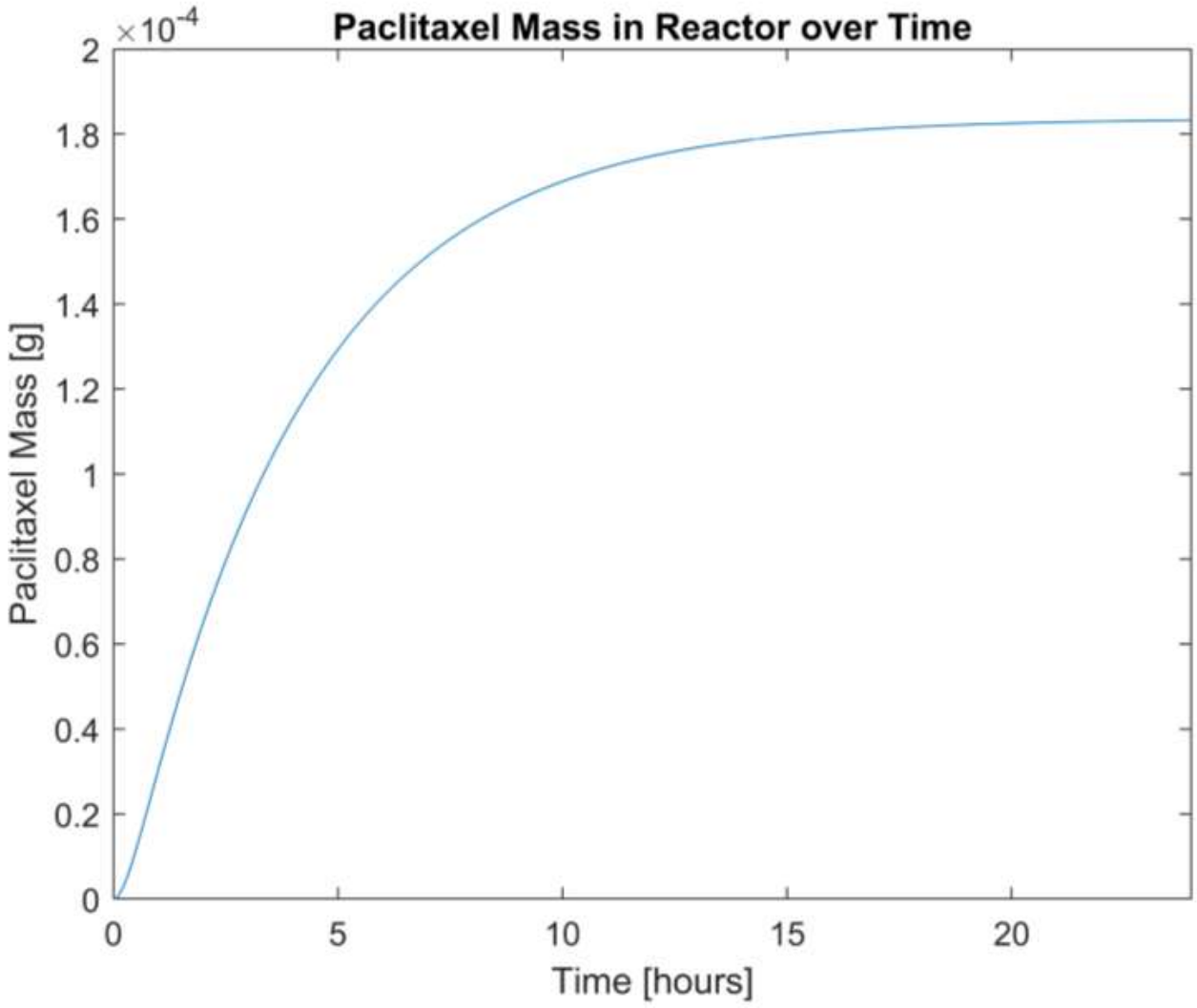

Figure 6: Graphical output of Microbial Consortium Model Iteration IV

MODEL EVALUATION

Assuming the cells do not divide, this model is relatively accurate. The next iteration will include cell growth and the use of resources to produce the cell growth.

APPENDIX A: TABLE OF NOMENCLATURE

Symbol	Parameter Meaning	Units
a	Concentration of acetate in the reactor	[g/L]
C	Concentration in a flow	[g/L]
d	Concentration of taxadiene in the reactor	[g/L]
E	Energy	[J]
e	Concentration of *E. coli* cells in the reactor	[g/L]
F	Flow rate	[L/h]
H	Heat of reaction	[J/mol]
m	Mass in a system	[g]
p	Concentration of paclitaxel in the reactor	[g/L]
r	Reaction rate	[mol/L-h]
s	Concentration of *S. cerevisiae* cells in the reactor	[g/L]
T	Temperature	[K]
t	Time	[h]
V	Volume of a system	[L]
W	Molecular weight	[g/mol]
x	Concentration of xylose in the reactor	[g/L]

Subscript	Meaning
1	Property of Flow 1
2	Property of Flow 2
3	Property of Flow 3
4	Property of Flow 4
5	Property of Flow 5
6	Property of Flow 6
7	Property of Flow 7
a	Property of acetate
a,s	Property of lumped reactions to convert acetate to *S. cerevisiae* cell growth
d	Property of taxadiene
d,p	Property of lumped reactions to convert taxadiene to paclitaxel
e	Property of *E. coli* cells
p	Property of paclitaxel
s	Property of *S. cerevisiae* cells
s1	Property of Subsystem 1
s2	Property of Subsystem 2
x,a	Property of lumped reactions to convert xylose to acetate
x,d	Property of lumped reactions to convert xylose to taxadiene
x,e	Property of lumped reactions to convert xylose to *E. coli* cell growth

APPENDIX B: SUPPLEMENTAL FIGURES

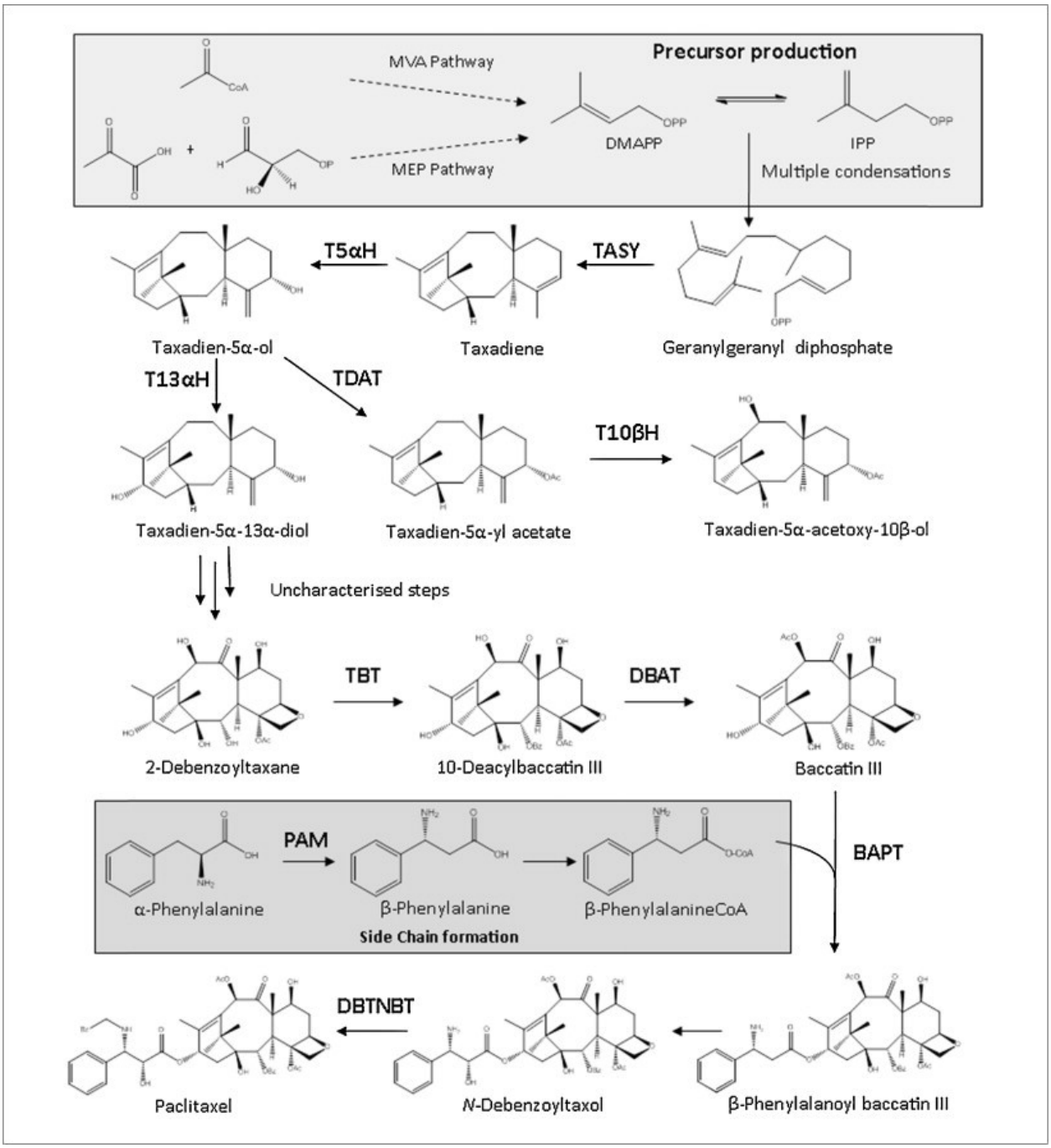

Figure 7: Paclitaxel biosynthesis pathway (Howat S. , et al., 2014). The first half of the pathway, up to the production of taxadiene, is performed in the E. coli *cell while the rest of the pathway is performed in the* S. cerevisiae *cell.*

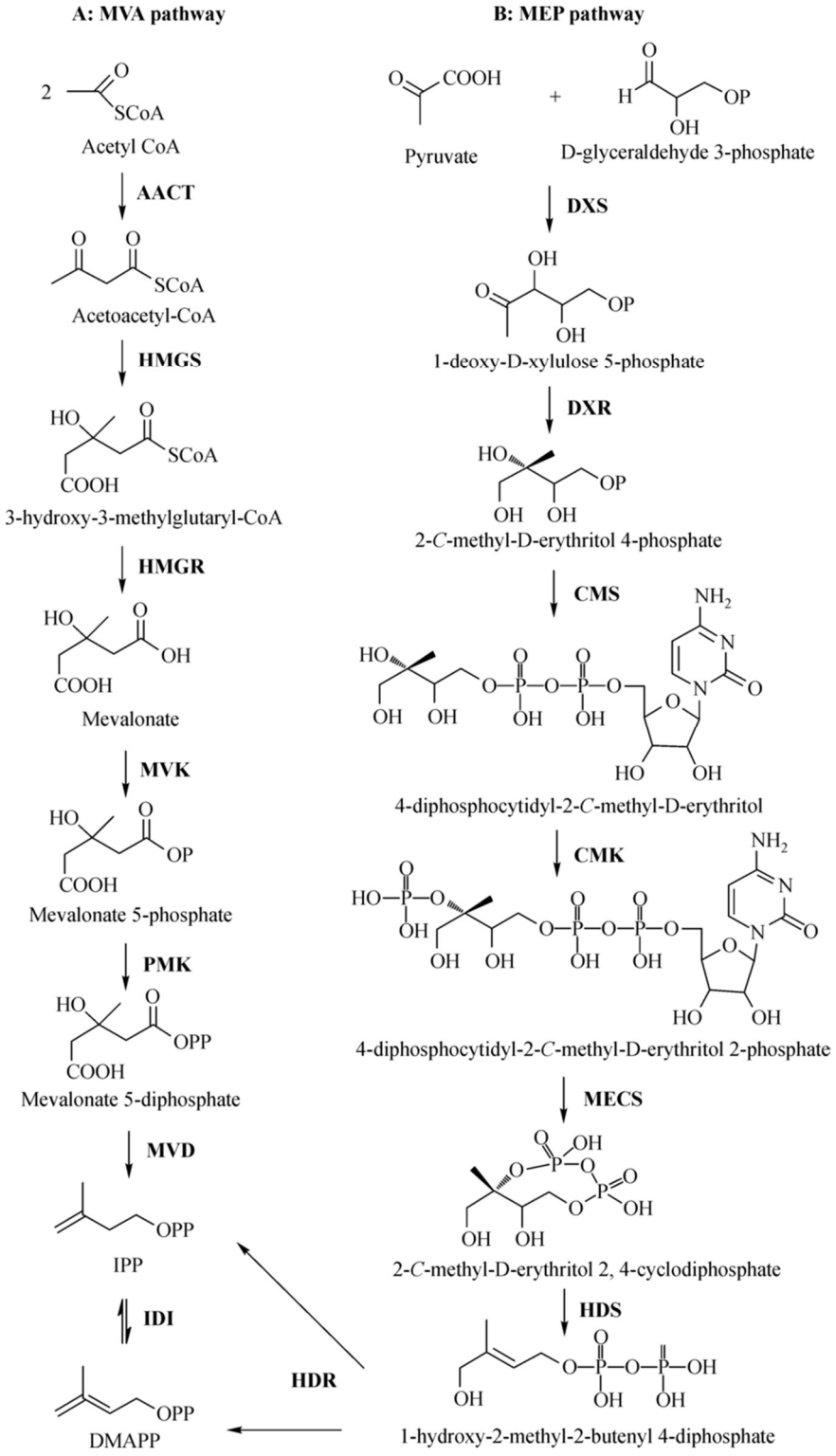

Figure 8: The MEV and MEP pathways referenced in Figure 7 (Zhu, Zeng, Sun, & Chen, 2014). These pathways are performed in the E. coli *cell.*

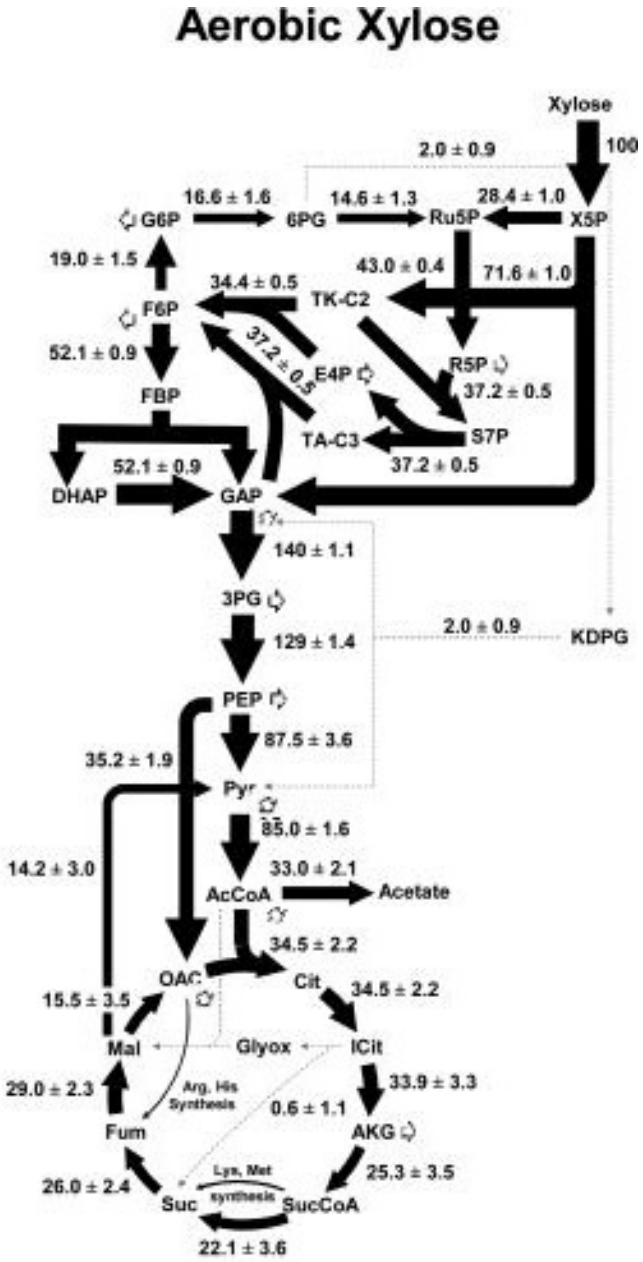

Figure 9: E. coli *aerobic metabolism of xylose (Gonzalez, Long, & Antoniewicz, 2017). The* E. coli *cell produces the acetate and then transports the molecule to the fermentation broth, where it is then taken up by* S. cerevisiae.

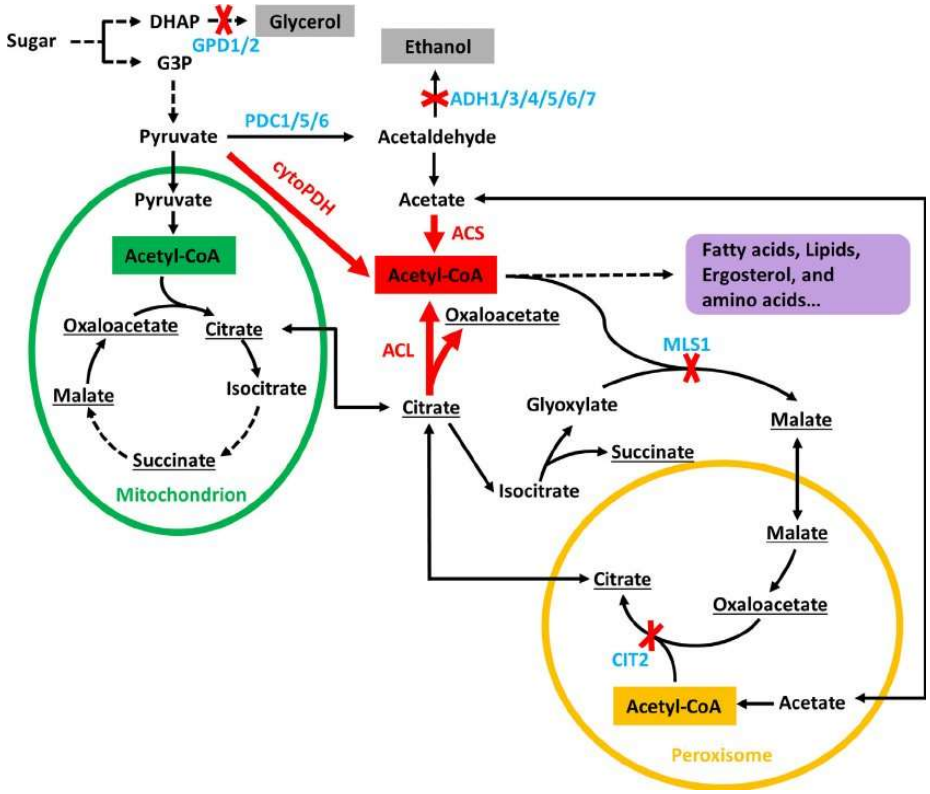

Figure 10: Metabolism of acetate in S. cerevisiae (Lian, Si, Nair, & Zhao, 2014). The acetate is produced in E. coli before being taken up by the S. cerevisiae and being incorporated into the metabolism.

APPENDIX C: MODEL CODE

ITERATION I

```
clear;
% Constants and Initial Conditions
F1 = 0; % [L/h]
C1 = 5; % [g/L]
F2 = 0; % [L/h]
V = 1; % [L]
T = 273 + 30; % [K]
cp = 4.186; % [J/g-K]
e = 2; % [g/L]
s = 2; % [g/L]
rho_cell = 200; % [g/L]
Vs1 = e * V / rho_cell; % [L]
Vs2 = s * V / rho_cell; % [L]
rho_water = 1000; % g/L
x = 5; % [g/L]
p = 0; % [g/L]
d = 0; % [g/L]
a = 0; % [g/L]
Wx = 150.13; % [g/mol]
Wd = 272.476; % [g/mol]
Wa = 60.052; % [g/mol]
Wp = 853.906; % [g/mol]
Hxd = 15; % [J/mol]
Hxa = 7; % [J/mol]
Hxe = 0; % [J/mol]
Hdp = 8; % [J/mol]
Has = 0; % [J/mol]
m = (e + s + x + p + d + a) * V; % [g]
ms1 = e; % [g]
ms2 = s; % [g]
time = 0:0.01:24; % [h]
p_t = zeros(length(time),1);
i = 1;
delt = 0.01;
for i = 1:length(time)
    p_t(i) = p; % [g]
    rxe = 0;
    rxd = 1 / (1/0.65 + 1/0.57 + 1/0.891 + 1/0.078 + 1/0.52 + 1/0.134 + 1/0.0003
+ 1/506 + 1/2 ...
        + 1/0.0035 + 1/0.06 + 1/0.06 + 1/0.00000133 + 1/0.109 + 1/3 + 1/1.6 +
1/23 + 1/33 ...
        + 1/0.75 + 1/0.099 + 1/0.03); % [mol/L-min]
    rxd = rxd * 60; % [mol/L-h]
    rxa = 1 / (1/0.65 + 1/0.57 + 1/0.891 + 1/0.078 + 1/0.52 + 1/0.134 + 1/0.885);
% [mol/L-min]
    rxa = rxa * 60; % [mol/L-h]
```

```
    rdp = 1 / (1/0.016 + 1/5.77 + 1/0.00635 + 1/6.1 + 1/2.2 + 1/0.0049 +
1/0.0049); % [mol/L-min]
    rdp = rdp * 60; % [mol/L-h]
    ras = 0;
    % integrate dxdt = F1 * C1 - (rxe + rxd + rxa) * Wx * Vs1; % [g/h]
    x = x + F1 * C1 * delt - (rxe + rxd + rxa) * Wx * Vs1 * delt; % [g]
    if x < 0
        x = 0;
    end
    % integrate dddt = rxd * Wx * Vs1 - rdp * Wd * Vs2; % [g/h]
    d = d + rxd * Wx * Vs1 * delt - rdp * Wd * Vs2 * delt; % [g]
    if d < 0
        d = 0;
    end
    % integrate dadt = rxa * Wx * Vs1 - ras * Wa * Vs2; % [g/h]
    a = a + rxa * Wx * Vs1 * delt - ras * Wa * Vs2 * delt; % [g]
    if a < 0
        a = 0;
    end
    % integrate dedt = rxe * Wx * Vs1; % [g/h]
    e = e + rxe * Wx * Vs1 * delt; % [g]
    if e < 0
        e = 0;
    end
    % integrate dsdt = ras * Wa * Vs2; % [g/h]
    s = s + ras * Wa * Vs2 * delt;
    if s < 0
        s = 0;
    end
    C2 = p / V; % [g/L]
    % integrate dpdt = rdp * Vs2 - F2 * C2
    p = p + rdp * Vs2 * Wd * delt - F2 * C2 * delt; % [g]
    if p < 0
        p = 0;
    end

    % integrate dmdt = F1 * C1 - F2 * C2; % [g/h]
    m = m + F1 * C1 * delt - F2 * C2 * delt; % [g]
    if m < 0
        m = 0;
    end
    % integrate dms1dt = F1 * C1 - Vs1 * Wx * (rxd + rxa); % [g/h]
    ms1 = ms1 + F1 * C1 * delt - Vs1 * Wx * (rxd + rxa) * delt; % [g]
    if ms1 < 0
        ms1 = 0;
    end
    % integrate dms2dt = Vs1 * Wx * (rxd + rxa) - F2 * C2; % [g/h]
    ms2 = ms2 + Vs1 * Wx * (rxd + rxa) * delt - F2 * C2 * delt; % [g]
    if ms2 < 0
        ms2 = 0;
```

```
    end

    % Assuming Subsystems Maintain a constant temperature
    % dhs1dt = Vs1 * (Hxd * Wd * rxd + Hxa * Wa * rxa + Hxe * We * rxe) - F6
    F6 = Vs1 * Wx * (Hxd * rxd + Hxa * rxa + Hxe * rxe);
    if F6 < 0
        F6 = 0;
    end
    % dhs2dt = Vs2 * (Hdp * Wp * rdp + Has * Ws * ras) - F7
    F7 = Vs2 * (Hdp * Wd * rdp + Has * Wa * ras);
    if F7 < 0
        F7 = 0;
    end

    % Assume F3 = 0
    F3 = 0; % [J/h]
    dhdt = F6 + F7 - F3;
    if dhdt < 0
        dhdt = 0;
    end
    T = T + dhdt / (e + s + (rho_water - (e + s)) * V * cp); % [K]
end
plot(time, p_t)
title('Paclitaxel Mass in Reactor over Time')
xlabel('Time [hours]')
xlim([0,24])
ylabel('Paclitaxel Mass [g]')
```

ITERATION II

```
clear;
% Constants and Initial Conditions
F1 = 0; % [L/h]
C1 = 5; % [g/L]
F2 = 0; % [L/h]
V = 1; % [L]
T = 273 + 30; % [K]
cp = 4.186; % [J/g-K]
e = 2; % [g/L]
s = 2; % [g/L]
rho_cell = 200; % [g/L]
Vs1 = e * V / rho_cell; % [L]
Vs2 = s * V / rho_cell; % [L]
rho_water = 1000; % g/L
x = 5; % [g/L]
p = 0; % [g/L]
d = 0; % [g/L]
a = 0; % [g/L]
Wx = 150.13; % [g/mol]
Wd = 272.476; % [g/mol]
Wa = 60.052; % [g/mol]
```

```
Wp = 853.906; % [g/mol]
Hxd = 15; % [J/mol]
Hxa = 7; % [J/mol]
Hxe = 0; % [J/mol]
Hdp = 8; % [J/mol]
Has = 0; % [J/mol]
m = (e + s + x + p + d + a) * V; % [g]
ms1 = e; % [g]
ms2 = s; % [g]
time = 0:0.01:24; % [h]
p_t = zeros(length(time),1);
i = 1;
delt = 0.01;
for i = 1:length(time)
    p_t(i) = p; % [g]
    rxe = 0;
    rxd = 1 / (1/0.65 + 1/0.57 + 1/0.891 + 1/0.078 + 1/0.52 + 1/0.134 + 1/0.0003
+ 1/506 + 1/2 ...
        + 1/0.0035 + 1/0.06 + 1/0.06 + 1/0.00000133 + 1/0.109 + 1/3 + 1/1.6 +
1/23 + 1/33 ...
        + 1/0.75 + 1/0.099 + 1/0.03); % [mol/L-min]
    rxd = rxd * 60; % [mol/L-h]
    rxa = 1 / (1/0.65 + 1/0.57 + 1/0.891 + 1/0.078 + 1/0.52 + 1/0.134 + 1/0.885);
% [mol/L-min]
    rxa = rxa * 60; % [mol/L-h]
    rdp = 1 / (1/0.016 + 1/5.77 + 1/0.00635 + 1/6.1 + 1/2.2 + 1/0.0049 +
1/0.0049); % [mol/L-min]
    rdp = rdp * 60; % [mol/L-h]
    if x < 272.5 / 6.02e23 % mass of one molecule of taxadiene
        rxd = 0;
    end
    if x < 60 / 6.02e23 % mass of one molecule of acetate
        rxa = 0;
    end
    if d < 853.9 / 6.02e23 % mass of one molecule of paclitaxel
        rdp = 0;
    end
    ras = 0;
    % integrate dxdt = F1 * C1 - (rxe + rxd + rxa) * Wx * Vs1; % [g/h]
    x = x + F1 * C1 * delt - (rxe + rxd + rxa) * Wx * Vs1 * delt; % [g]
    if x < 0
        x = 0;
    end
    % integrate dddt = rxd * Wx * Vs1 - rdp * Wd * Vs2; % [g/h]
    d = d + rxd * Wx * Vs1 * delt - rdp * Wd * Vs2 * delt; % [g]
    if d < 0
        d = 0;
    end
    % integrate dadt = rxa * Wx * Vs1 - ras * Wa * Vs2; % [g/h]
    a = a + rxa * Wx * Vs1 * delt - ras * Wa * Vs2 * delt; % [g]
```

```
    if a < 0
        a = 0;
    end
    % integrate dedt = rxe * Wx * Vs1; % [g/h]
    e = e + rxe * Wx * Vs1 * delt; % [g]
    if e < 0
        e = 0;
    end
    % integrate dsdt = ras * Wa * Vs2; % [g/h]
    s = s + ras * Wa * Vs2 * delt;
    if s < 0
        s = 0;
    end
    C2 = p / V; % [g/L]
    % integrate dpdt = rdp * Vs2 - F2 * C2
    p = p + rdp * Vs2 * Wd * delt - F2 * C2 * delt; % [g]
    if p < 0
        p = 0;
    end

    % integrate dmdt = F1 * C1 - F2 * C2; % [g/h]
    m = m + F1 * C1 * delt - F2 * C2 * delt; % [g]
    if m < 0
        m = 0;
    end
    % integrate dms1dt = F1 * C1 - Vs1 * Wx * (rxd + rxa); % [g/h]
    ms1 = ms1 + F1 * C1 * delt - Vs1 * Wx * (rxd + rxa) * delt; % [g]
    if ms1 < 0
        ms1 = 0;
    end
    % integrate dms2dt = Vs1 * Wx * (rxd + rxa) - F2 * C2; % [g/h]
    ms2 = ms2 + Vs1 * Wx * (rxd + rxa) * delt - F2 * C2 * delt; % [g]
    if ms2 < 0
        ms2 = 0;
    end

    % Assuming Subsystems Maintain a constant temperature
    % dhs1dt = Vs1 * (Hxd * Wd * rxd + Hxa * Wa * rxa + Hxe * We * rxe) - F6
    F6 = Vs1 * Wx * (Hxd * rxd + Hxa * rxa + Hxe * rxe);
    if F6 < 0
        F6 = 0;
    end
    % dhs2dt = Vs2 * (Hdp * Wp * rdp + Has * Ws * ras) - F7
    F7 = Vs2 * (Hdp * Wd * rdp + Has * Wa * ras);
    if F7 < 0
        F7 = 0;
    end

    % Assume F3 = 0
    F3 = 0; % [J/h]
```

```
    dhdt = F6 + F7 - F3;
    if dhdt < 0
        dhdt = 0;
    end
    T = T + dhdt / (e + s + (rho_water - (e + s)) * V * cp); % [K]
end
plot(time, p_t)
title('Paclitaxel Mass in Reactor over Time')
xlabel('Time [hours]')
xlim([0,24])
ylabel('Paclitaxel Mass [g]')
```

ITERATION III

```
clear;
% Constants and Initial Conditions
F1 = 0; % [L/h]
C1 = 5; % [g/L]
F2 = 0; % [L/h]
V = 1; % [L]
T = 273 + 30; % [K]
cp = 4.186; % [J/g-K]
e = 2; % [g/L]
s = 2; % [g/L]
rho_cell = 200; % [g/L]
Vs1 = e * V / rho_cell; % [L]
Vs2 = s * V / rho_cell; % [L]
rho_water = 1000; % g/L
x = 5; % [g/L]
p = 0; % [g/L]
d = 0; % [g/L]
a = 0; % [g/L]
Wx = 150.13; % [g/mol]
Wd = 272.476; % [g/mol]
Wa = 60.052; % [g/mol]
Wp = 853.906; % [g/mol]
Hxd = 15; % [J/mol]
Hxa = 7; % [J/mol]
Hxe = 0; % [J/mol]
Hdp = 8; % [J/mol]
Has = 0; % [J/mol]
m = (e + s + x + p + d + a) * V; % [g]
ms1 = e; % [g]
ms2 = s; % [g]
time = 0:0.01:24; % [h]
p_t = zeros(length(time),1);
i = 1;
delt = 0.01;
for i = 1:length(time)
    p_t(i) = p; % [g]
    rxe = 0 * x;
```

```
    rxd = x * 1 / (1/0.65 + 1/0.57 + 1/0.891 + 1/0.078 + 1/0.52 + 1/0.134 +
1/0.0003 + 1/506 + 1/2 ...
        + 1/0.0035 + 1/0.06 + 1/0.06 + 1/0.00000133 + 1/0.109 + 1/3 + 1/1.6 +
1/23 + 1/33 + 1/0.75 ...
        + 1/0.099 + 1/0.03); % [mol/L-min]
    rxd = rxd * 60; % [mol/L-h]
    rxa = x * 1 / (1/0.65 + 1/0.57 + 1/0.891 + 1/0.078 + 1/0.52 + 1/0.134 +
1/0.885); % [mol/L-min]
    rxa = rxa * 60; % [mol/L-h]
    rdp = d * 1 / (1/0.016 + 1/5.77 + 1/0.00635 + 1/6.1 + 1/2.2 + 1/0.0049 +
1/0.0049); % [mol/L-min]
    rdp = rdp * 60; % [mol/L-h]
    if x < 272.5 / 6.02e23 % mass of one molecule of taxadiene
        rxd = 0;
    end
    if x < 60 / 6.02e23 % mass of one molecule of acetate
        rxa = 0;
    end
    if d < 853.9 / 6.02e23 % mass of one molecule of paclitaxel
        rdp = 0;
    end
    ras = 0 * a;
    % integrate dxdt = F1 * C1 - (rxe + rxd + rxa) * Wx * Vs1; % [g/h]
    x = x + F1 * C1 * delt - (rxe + rxd + rxa) * Wx * Vs1 * delt; % [g]
    if x < 0
        x = 0;
    end
    % integrate dddt = rxd * Wx * Vs1 - rdp * Wd * Vs2; % [g/h]
    d = d + rxd * Wx * Vs1 * delt - rdp * Wd * Vs2 * delt; % [g]
    if d < 0
        d = 0;
    end
    % integrate dadt = rxa * Wx * Vs1 - ras * Wa * Vs2; % [g/h]
    a = a + rxa * Wx * Vs1 * delt - ras * Wa * Vs2 * delt; % [g]
    if a < 0
        a = 0;
    end
    % integrate dedt = rxe * Wx * Vs1; % [g/h]
    e = e + rxe * Wx * Vs1 * delt; % [g]
    if e < 0
        e = 0;
    end
    % integrate dsdt = ras * Wa * Vs2; % [g/h]
    s = s + ras * Wa * Vs2 * delt;
    if s < 0
        s = 0;
    end
    C2 = p / V; % [g/L]
    % integrate dpdt = rdp * Vs2 - F2 * C2
    p = p + rdp * Vs2 * Wd * delt - F2 * C2 * delt; % [g]
```

```
    if p < 0
        p = 0;
    end

    % integrate dmdt = F1 * C1 - F2 * C2; % [g/h]
    m = m + F1 * C1 * delt - F2 * C2 * delt; % [g]
    if m < 0
        m = 0;
    end
    % integrate dms1dt = F1 * C1 - Vs1 * Wx * (rxd + rxa); % [g/h]
    ms1 = ms1 + F1 * C1 * delt - Vs1 * Wx * (rxd + rxa) * delt; % [g]
    if ms1 < 0
        ms1 = 0;
    end
    % integrate dms2dt = Vs1 * Wx * (rxd + rxa) - F2 * C2; % [g/h]
    ms2 = ms2 + Vs1 * Wx * (rxd + rxa) * delt - F2 * C2 * delt; % [g]
    if ms2 < 0
        ms2 = 0;
    end

    % Assuming Subsystems Maintain a constant temperature
    % dhs1dt = Vs1 * (Hxd * Wd * rxd + Hxa * Wa * rxa + Hxe * We * rxe) - F6
    F6 = Vs1 * Wx * (Hxd * rxd + Hxa * rxa + Hxe * rxe);
    if F6 < 0
        F6 = 0;
    end
    % dhs2dt = Vs2 * (Hdp * Wp * rdp + Has * Ws * ras) - F7
    F7 = Vs2 * (Hdp * Wd * rdp + Has * Wa * ras);
    if F7 < 0
        F7 = 0;
    end

    % Assume F3 = 0
    F3 = 0; % [J/h]
    dhdt = F6 + F7 - F3;
    if dhdt < 0
        dhdt = 0;
    end
    T = T + dhdt / (e + s + (rho_water - (e + s)) * V * cp); % [K]
end
plot(time, p_t)
title('Paclitaxel Mass in Reactor over Time')
xlabel('Time [hours]')
xlim([0,24])
ylabel('Paclitaxel Mass [g]')
```

ITERATION IV

```
clear;
% Constants and Initial Conditions
F1 = 0; % [L/h]
```

```
C1 = 5; % [g/L]
F2 = 0; % [L/h]
V = 1; % [L]
T = 273 + 30; % [K]
cp = 4.186; % [J/g-K]
e = 2; % [g/L]
s = 2; % [g/L]
rho_cell = 200; % [g/L]
Vs1 = e * V / rho_cell; % [L]
Vs2 = s * V / rho_cell; % [L]
rho_water = 1000; % g/L
x = 5; % [g/L]
p = 0; % [g/L]
d = 0; % [g/L]
a = 0; % [g/L]
Wx = 150.13; % [g/mol]
Wd = 272.476; % [g/mol]
Wa = 60.052; % [g/mol]
Wp = 853.906; % [g/mol]
Hxd = 15; % [J/mol]
Hxa = 7; % [J/mol]
Hxe = 0; % [J/mol]
Hdp = 8; % [J/mol]
Has = 0; % [J/mol]
m = (e + s + x + p + d + a) * V; % [g]
ms1 = e; % [g]
ms2 = s; % [g]
time = 0:0.01:24; % [h]
p_t = zeros(length(time),1);
i = 1;
delt = 0.01;
for i = 1:length(time)
    p_t(i) = p; % [g]
    rxe = 0 * x;
    rxd = x * 0.5 * 1 / (1/0.65 + 1/0.57 + 1/0.891 + 1/0.078 + 1/0.52 + 1/0.134 +
1/0.0003 + 1/506 + 1/2 ...
        + 1/0.0035 + 1/0.06 + 1/0.06 + 1/0.00000133 + 1/0.109 + 1/3 + 1/1.6 +
1/23 + 1/33 + 1/0.75 + ...
        1/0.099 + 1/0.03); % [mol/L-min]
    rxd = rxd * 60; % [mol/L-h]
    rxa = x * 0.5 * 1 / (1/0.65 + 1/0.57 + 1/0.891 + 1/0.078 + 1/0.52 + 1/0.134 +
1/0.885); % [mol/L-min]
    rxa = rxa * 60; % [mol/L-h]
    rdp = d *1 / (1/0.016 + 1/5.77 + 1/0.00635 + 1/6.1 + 1/2.2 + 1/0.0049 +
1/0.0049); % [mol/L-min]
    rdp = rdp * 60; % [mol/L-h]
    if x < 272.5 / 6.02e23 % mass of one molecule of taxadiene
        rxd = 0;
    end
    if x < 60 / 6.02e23 % mass of one molecule of acetate
```

```
    rxa = 0 * a;
end
if d < 853.9 / 6.02e23 % mass of one molecule of paclitaxel
    rdp = 0;
end
ras = 0 * a;
% integrate dxdt = F1 * C1 - (rxe + rxd + rxa) * Wx * Vs1; % [g/h]
x = x + F1 * C1 * delt - (rxe + rxd + rxa) * Wx * Vs1 * delt; % [g]
if x < 0
    x = 0;
end
% integrate dddt = rxd * Wx * Vs1 - rdp * Wd * Vs2; % [g/h]
d = d + rxd * Wx * Vs1 * delt - rdp * Wd * Vs2 * delt; % [g]
if d < 0
    d = 0;
end
% integrate dadt = rxa * Wx * Vs1 - ras * Wa * Vs2; % [g/h]
a = a + rxa * Wx * Vs1 * delt - ras * Wa * Vs2 * delt; % [g]
if a < 0
    a = 0;
end
% integrate dedt = rxe * Wx * Vs1; % [g/h]
e = e + rxe * Wx * Vs1 * delt; % [g]
if e < 0
    e = 0;
end
% integrate dsdt = ras * Wa * Vs2; % [g/h]
s = s + ras * Wa * Vs2 * delt;
if s < 0
    s = 0;
end
C2 = p / V; % [g/L]
% integrate dpdt = rdp * Vs2 - F2 * C2
p = p + rdp * Vs2 * Wd * delt - F2 * C2 * delt; % [g]
if p < 0
    p = 0;
end

% integrate dmdt = F1 * C1 - F2 * C2; % [g/h]
m = m + F1 * C1 * delt - F2 * C2 * delt; % [g]
if m < 0
    m = 0;
end
% integrate dms1dt = F1 * C1 - Vs1 * Wx * (rxd + rxa); % [g/h]
ms1 = ms1 + F1 * C1 * delt - Vs1 * Wx * (rxd + rxa) * delt; % [g]
if ms1 < 0
    ms1 = 0;
end
% integrate dms2dt = Vs1 * Wx * (rxd + rxa) - F2 * C2; % [g/h]
ms2 = ms2 + Vs1 * Wx * (rxd + rxa) * delt - F2 * C2 * delt; % [g]
```

```
    if ms2 < 0
        ms2 = 0;
    end

    % Assuming Subsystems Maintain a constant temperature
    % dhs1dt = Vs1 * (Hxd * Wd * rxd + Hxa * Wa * rxa + Hxe * We * rxe) - F6
    F6 = Vs1 * Wx * (Hxd * rxd + Hxa * rxa + Hxe * rxe);
    if F6 < 0
        F6 = 0;
    end
    % dhs2dt = Vs2 * (Hdp * Wp * rdp + Has * Ws * ras) - F7
    F7 = Vs2 * (Hdp * Wd * rdp + Has * Wa * ras);
    if F7 < 0
        F7 = 0;
    end

    % Assume F3 = 0
    F3 = 0; % [J/h]
    dhdt = F6 + F7 - F3;
    if dhdt < 0
        dhdt = 0;
    end
    T = T + dhdt / (e + s + (rho_water - (e + s)) * V * cp); % [K]
end
plot(time, p_t)
title('Paclitaxel Mass in Reactor over Time')
xlabel('Time [hours]')
xlim([0,24])
ylabel('Paclitaxel Mass [g]')
```

APPENDIX D: REFERENCES

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