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| ABE 30100 |
| Microbial Consortium Modeling |
| Deliverable III |

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| Kathryn Atherton  3-18-2019 |

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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).

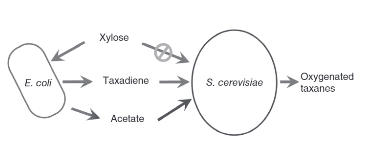


Figure 1: Picture of the fermentation process to be modeled. The E. coli consumes xylose and produces acetate for the S. cerevisiae to uses as a carbon source. E. coli produce taxadiene for the S. cerevisiae to oxygenate and use to produce the final product, paclitaxel *(Zhou, Qiao, Edgar, & Stephanopoulos, 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

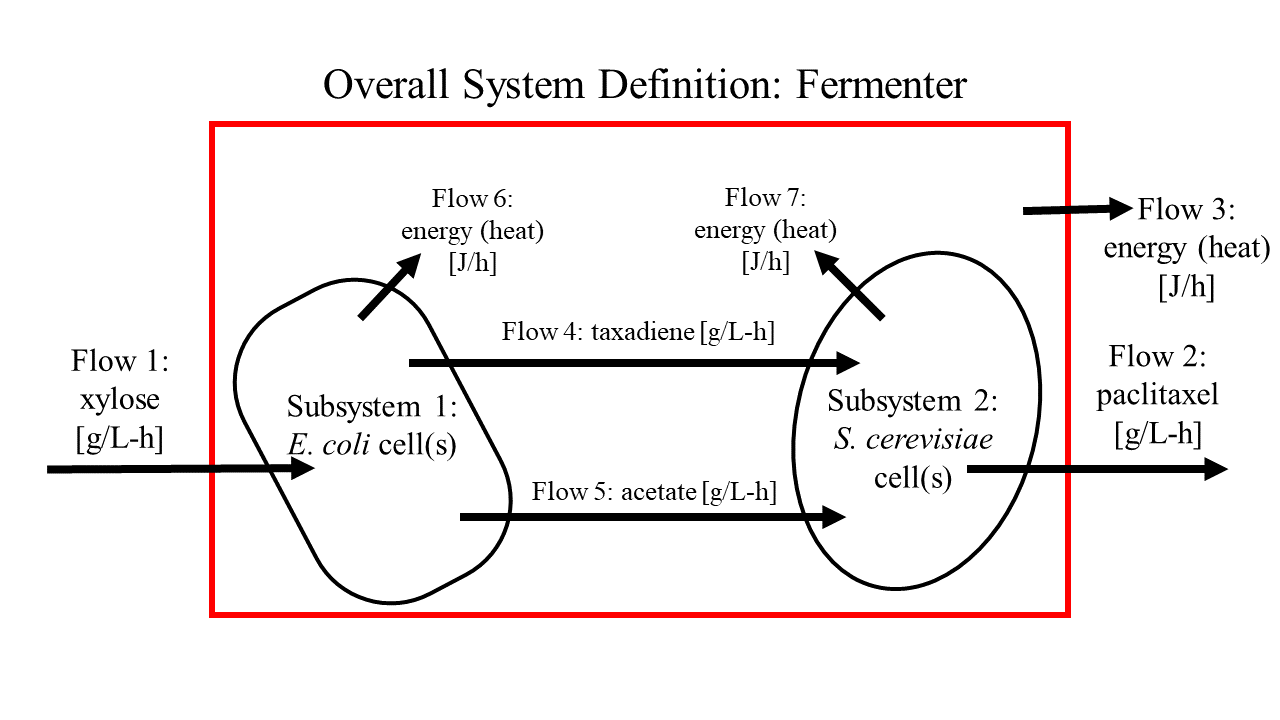


Figure 2: System definition with input and output flows.

## 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

|  |  |  |
| --- | --- | --- |
|  |  | [*1*] |

Unit Analysis:

#### 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

|  |  |  |
| --- | --- | --- |
|  |  | [*2*] |

Unit Analysis:

#### 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

|  |  |  |
| --- | --- | --- |
|  |  | [*3*] |

Unit Analysis:

### 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

|  |  |  |
| --- | --- | --- |
|  |  | [*4*] |

Unit Analysis:

* Note: The consumption of xylose to produce cell growth (rx,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

|  |  |  |
| --- | --- | --- |
|  |  | [*5*] |

Unit Analysis:

#### 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

|  |  |  |
| --- | --- | --- |
|  |  | [*6*] |

Unit Analysis:

#### 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

|  |  |  |
| --- | --- | --- |
|  |  | [*7*] |

Unit Analysis:

#### *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

|  |  |  |
| --- | --- | --- |
|  |  | [*8*] |

Unit Analysis:

* 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

|  |  |  |
| --- | --- | --- |
|  |  | [*9*] |

Unit Analysis:

* 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

|  |  |  |
| --- | --- | --- |
|  |  | [*10*] |

Unit Analysis:

#### 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

|  |  |  |
| --- | --- | --- |
|  |  | [*11*] |

Unit Analysis:

#### 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

|  |  |  |
| --- | --- | --- |
|  |  | [*12*] |

Unit Analysis:

## Relevant Parameters, Relationships, and Principles

### Parameters

* See Appendix A for parameter nomenclature and descriptions

### Relationships

* + This is used to determine the reaction rates based on the concentration(s) of the reactant(s)
  + 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
  + 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).

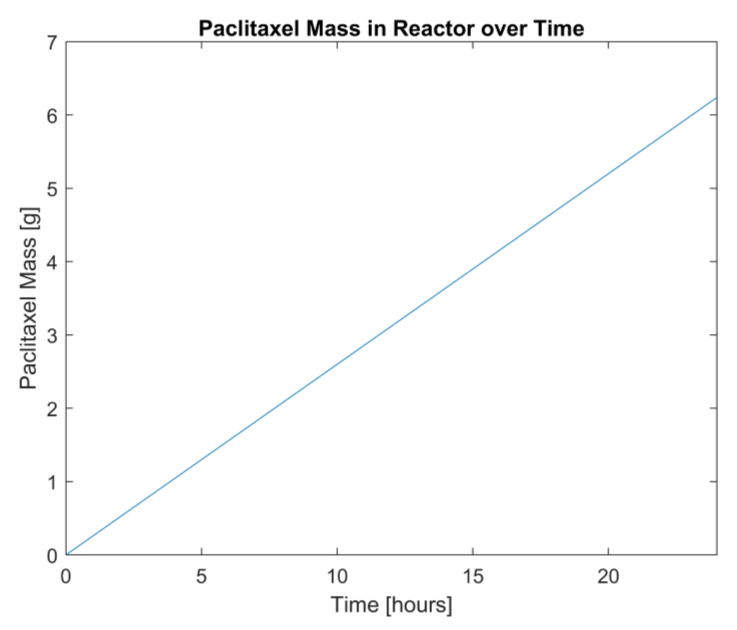


Figure 3: Graphical output of Microbial Consortium Model Iteration I

### 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.

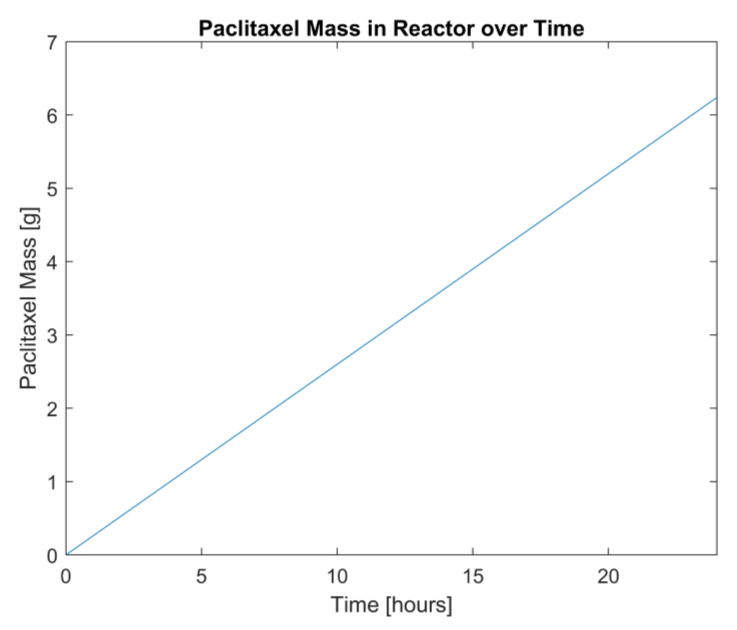


Figure 4: Graphical output of Microbial Consortium Model Iteration II

### 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.

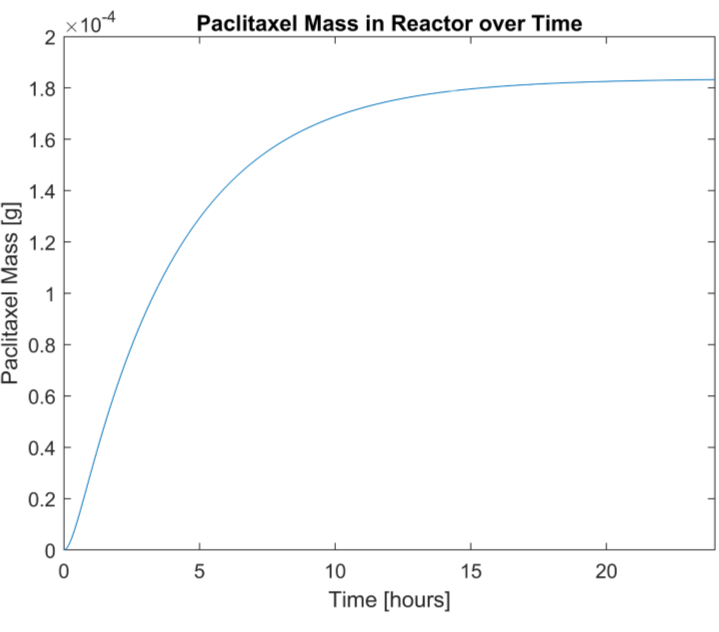


Figure 5: Graphical output of Microbial Consortium Model Iteration III

### 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.

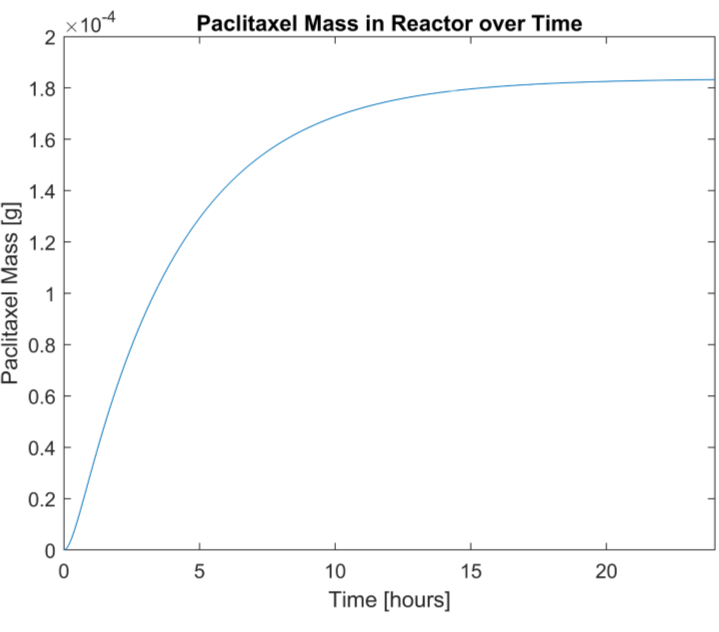


Figure 6: Graphical output of Microbial Consortium Model Iteration IV

### 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

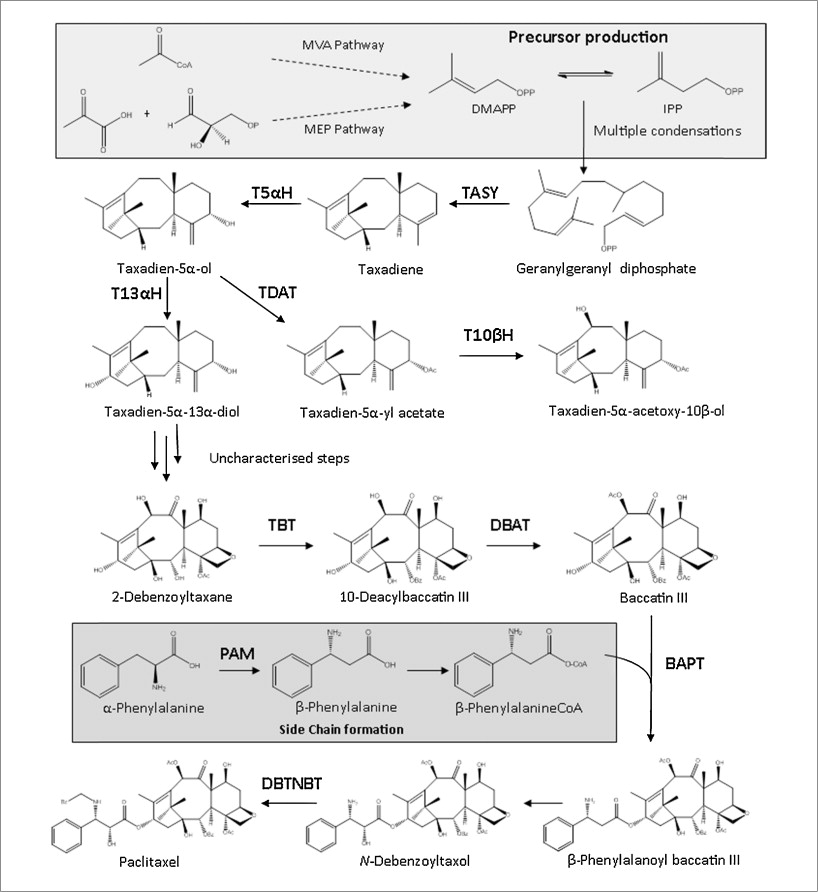


Figure 7: Paclitaxel biosynthesis pathway *(Howat S. , et al., 2014)*. The first half of the pathway, up to the production of taxadiene, is performed in the E. coli cell while the rest of the pathway is performed in the S. cerevisiae cell.

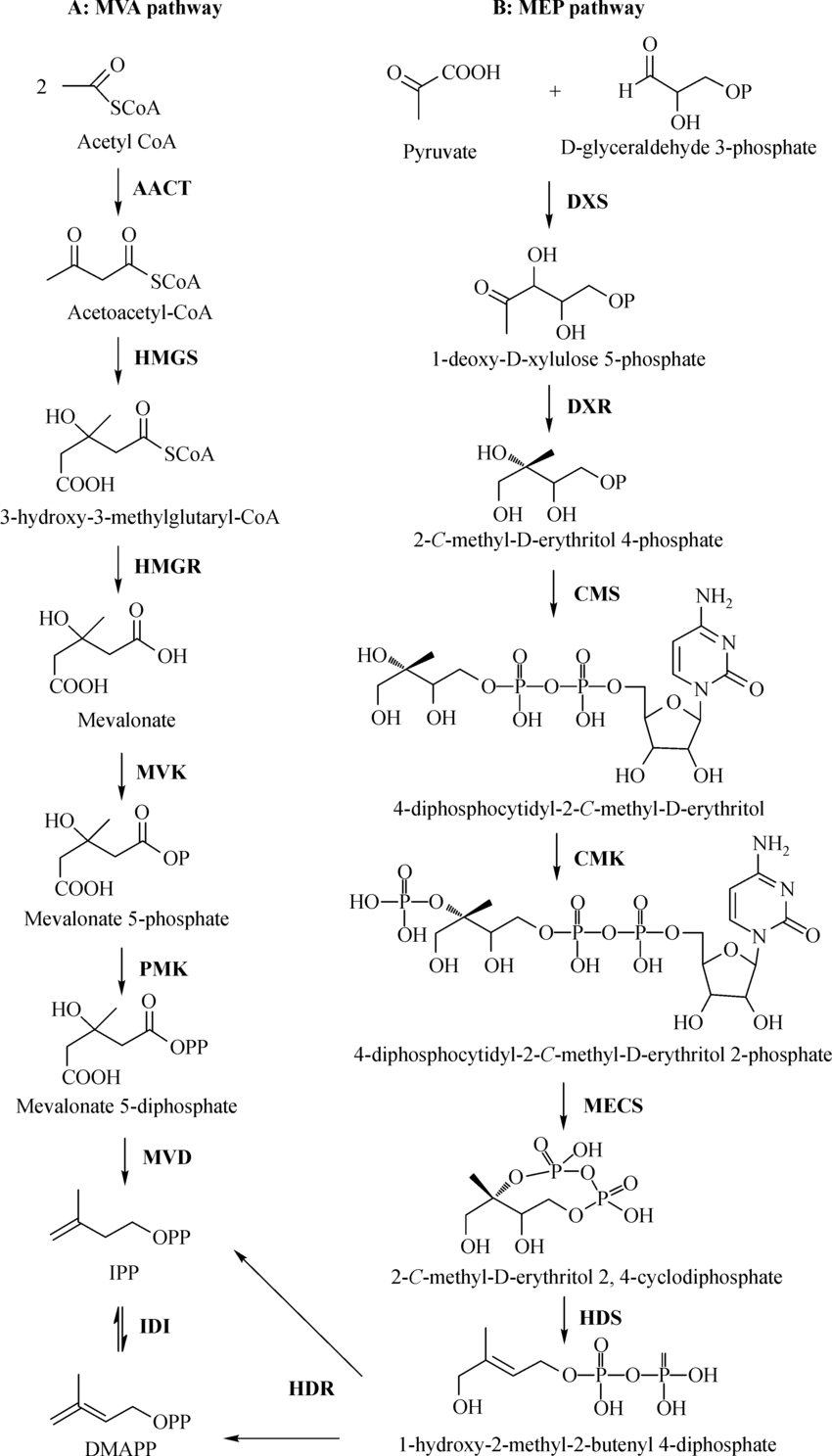


Figure 8: The MEV and MEP pathways referenced in Figure 7 *(Zhu, Zeng, Sun, & Chen, 2014)*. These pathways are performed in the E. coli cell.

An external file that holds a picture, illustration, etc.
Object name is nihms940987f6.jpg

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.

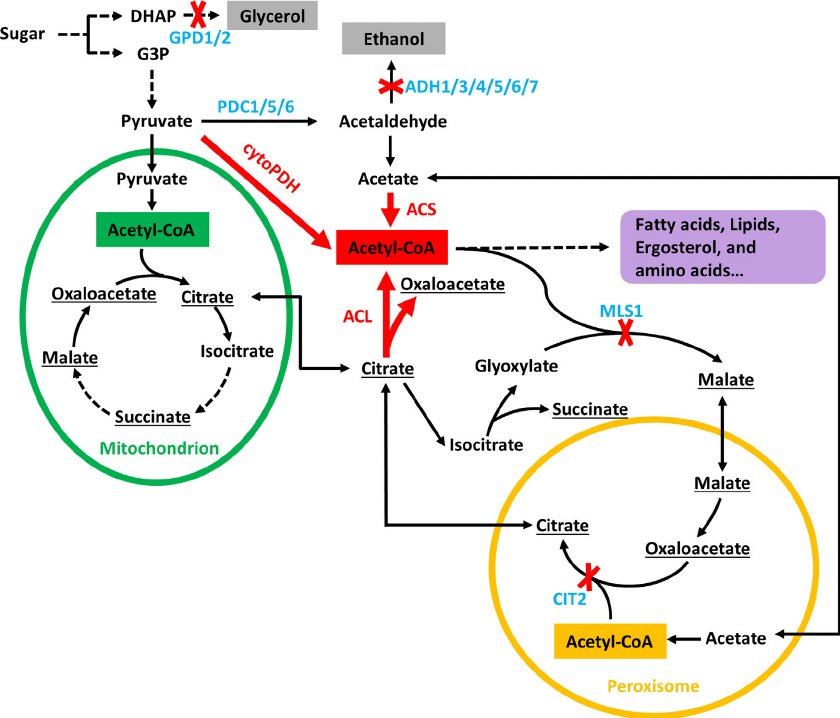


Figure 10: Metabolism of acetate in S. cerevisiae *(Lian, Si, Nair, & Zhao, 2014)*. The acetate is produced in E. coli before being taken up by the S. cerevisiae and being incorporated into the metabolism.

# 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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