Two Bacteria with Opposite Substrate Preferences (COMMENSA)

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System

Here is presented the batch growth of a two-organism culture on two substrates, in which both species can utilize both substrates (Kim et al., 1988), but where the organisms have opposing substrate preferences.

The two bacterial species involved are: *Klebsiella oxytoca* (XA) and *Pseudomonas aeruginosa* (XB). The two substrates are glucose (Y), which is preferred by *K. oxytoca,* and citrate (Z), which is preferred by *P. aeruginosa.*

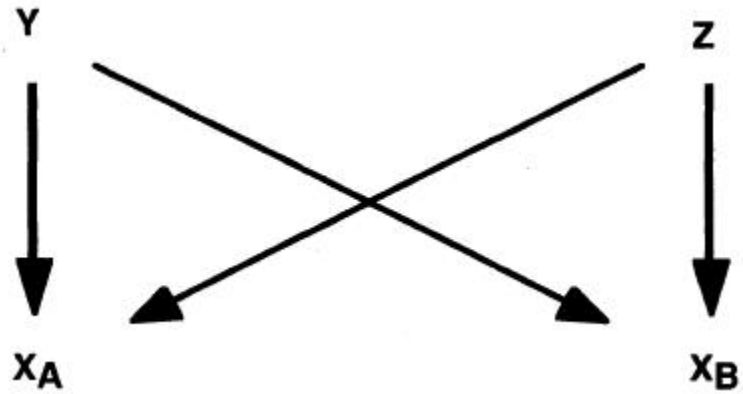

Fig. 1. Organism X_A prefers substrate Y, and organism X_B prefers substrate Z.

Model Assumptions

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- The overall individual growth rate of each species at any time is the sum of the rate of growth on glucose plus the rate on citrate.
- The specific growth rate on each substrate depends on the concentration level of some key enzyme responsible for the rate-controlling step E.
- An inhibitor I is produced from the growth of *K. oxytoca* (Bacteria-A) on glucose and inhibits the growth of *P. aeruginosa* (Bacteria-B) on citrate. The inhibitor is thus a growth-associated product.

Model

The growth rates, μ_{ij}, for each organism are the sums of the growth rates on glucose and citrate. The subscripts i and j have the following meaning: i refers to the organisms *(K. oxytoca* - A and P. *aeruginosa* = B) and j refers to the substrate (glucose = Y and citrate = Z). The levels of the key enzymes are denoted by E.

The biomass balances for the batch system are

$$\frac{dX_A}{dt} = (\mu_{AY} + \mu_{AZ})\, X_A$$

$$\frac{dX_B}{dt} = (\mu_{BY} + \mu_{BZ})\, X_B$$

Model

The specific growth rate equations for the two organisms on each substrate are given by :

$$\mu_{AY} = \frac{\mu_{maxAY} S_Y E_{AY}}{K_{SAY} + S_Y}$$

$$\mu_{AZ} = \frac{\mu_{maxAZ} S_Z E_{AZ}}{K_{SAZ} + S_Z}$$

$$\mu_{BY} = \frac{\mu_{maxBY} S_Y E_{BY}}{K_{SBY} + S_Y}$$

$$\mu_{BZ} = \frac{\mu_{maxBZ} S_Z E_{BZ}}{K_{SBZ} + S_Z} \left(\frac{K_I}{K_I + I} \right)$$

The substrate balances are given by :

$$\frac{dS_Y}{dt} = -\mu_{AY} X_A \left(\frac{1}{Y_{SAY}} + \frac{\alpha}{Y_I} \right) - \frac{\mu_{BY}}{Y_{SBY}} X_B$$

$$\frac{dS_Z}{dt} = -\frac{\mu_{BZ}}{Y_{SBZ}} X_B - \frac{\mu_{AZ}}{Y_{SAZ}} X_A$$

Model

Inhibitor (I) production is growth associated to organism A, and its decay is proportional to the cell concentration. The balance for the inhibitor is

$$\frac{dI}{dt} = \alpha \mu_{AY} X_A - \beta X_A$$

The balances for the key enzymes, which control the growth on secondary substrates are given by :

$$\frac{dE_{AZ}}{dt} = k_{PAZ} \frac{S_Z}{K_{EAZ}+S_Z} \frac{K_{RAZ}}{K_{RAZ}+S_Z} - k_{PAZ} E_{AZ}$$

and

$$\frac{dE_{BY}}{dt} = k_{PBY} \frac{S_Y}{K_{EBY}+S_Y} \frac{K_{RBY}}{K_{RBY}+S_Y} - k_{PBY} E_{BY}$$

Model

The oxygen uptake rate (OUR), carbon dioxide evolution rate (CER) and dissolved oxygen tension (DOT) are given by :

$$OUR = \left(\frac{\mu_{AY}}{Y_{OAY}} + \frac{\mu_{AZ}}{Y_{OAZ}} + m_{OAY} + m_{OAZ}\right)X_A + \left(\frac{\mu_{BY}}{Y_{OBY}} + \frac{\mu_{BZ}}{Y_{OBZ}} + m_{OBY} + m_{OBZ}\right)X_B$$

$$CER = \left(\frac{\mu_{AY}}{Y_{CAY}} + \frac{\mu_{AZ}}{Y_{CAZ}} + m_{CAY} + m_{CAZ}\right)X_A + \left(\frac{\mu_{BY}}{Y_{CBY}} + \frac{\mu_{BZ}}{Y_{CBZ}} + m_{CBY} + m_{CBZ}\right)X_B$$

$$DOT = 100\left(1 - \frac{OUR}{K_L a C_0^*}\right)$$

The cell mass fractions are given by :

$$F_A = \frac{X_A}{X_A + X_B}$$

$$F_B = 1 - F_A$$

Program

Function file

```
function dy = commensa_func(t,y,UMAY,UMAZ,UMBY,UMBZ,KSAY,KSAZ,KSBY,KSBZ,YSAY,YSAZ,YSBY,YSBZ,YOAY,YOAZ,YOBY,YOBZ,
dy = zeros(7,1);

UAY = UMAY*y(6)*EAY/(KSAY+y(6));
UAZ = UMAZ*y(7)*y(4)/(KSAZ+y(7));
UBY = UMBY*y(6)*y(5)/(KSBY+y(6));
UBZ = (KI*UMBZ*y(7)*EBZ)/((KSBZ + y(7))*(KI + y(3)));

dy(1) = (UAY + UAZ)*y(1);
dy(2) = (UBY + UBZ)*y(2);
dy(3) = ALPHA*UAY*y(1) - BETA*y(1);
if (y(3)<0)
    y(3)=0; end;
dy(4) = (KPAZ*y(7)*KRAZ)/((KEAZ + y(7))*(KRAZ + y(7))) - (KPAZ*y(4));
dy(5) = (KPBY*y(6)*KRBY)/((KEBY + y(6))*(KRBY + y(6))) - (KPBY*y(5));
dy(6) = (-UAY*y(1))*(1/(YSAY) + (ALPHA/YI))-y(2)*(UBY/YSBY);
if (y(6)<0)
    y(6)=0; end;
dy(7) = -y(2)*(UBZ/YSBZ) - y(1)*(UAZ/YSAZ);
if (y(7)<0)
    y(7)=0; end;
```

Program

Command File

```
1  -    UMAY = 0.933;          UMAZ = 0.926;
2  -    UMBY = 0.497;          UMBZ = 0.532;
3  -    KSAY = 0.004;          KSAZ = 0.001;
4  -    KSBY = 0.004;          KSBZ = 0.001;
5  -    YSAY = 0.503;          YSAZ = 0.3;
6  -    YSBY = 0.33;           YSBZ = 0.37;
7  -    YOAY = 2.25;           YOAZ = 2.32;
8  -    YOBY = 1.58;           YOBZ = 2.1;
9  -    YCAY = 1.6;            YCAZ = 0.9;
10 -    YCBY = 0.85;           YCBZ = 0.93;
11 -    YI = 0.05;
12 -    KPAZ = 0.4;            KPBY = 0.12;
13 -    ALPHA = 0.7;           BETA = 0.001;
14 -    MOAY = 0.0;            MOAZ = 0.1;
15 -    MOBY = 0.06;           MOBZ = 0.0;
16 -    MCAY = 0.11;           MCAZ = 0.08;
17 -    MCBY = 0.1;            MCBZ = 0.0;
18 -    KI = 0.0001;
19 -    EAY = 1.0;             EBZ = 1.0;
20 -    KEAZ = 0.001;          KEBY = 0.001;
21 -    KRAZ = 0.00001;        KRBY = 0.3;
22 -    KLACO = 0.585;
23 -    ALGO = 0.0;
```

Program

Command File

```
29 -    XA0 = 0.001;          XB0 = 0.01;
30 -    I0 = 0.0;
31 -    EAZ0 = 0.1;           EBY0 = 0.1;
32 -    SY0 = 0.74;           SZ0 = 0.8;
33 -    Cinit = [XA0, XB0, I0, EAZ0, EBY0, SY0, SZ0];
34
35 -    Tint = 0.1;
36 -    Tfin = 8.2;
37 -    Tspan = linspace(0,Tfin,Tfin/Tint);
38
39 -    [T C] = ode45(@(t,y) commensa_func(t,y,UMAY,UMAZ,UMBY,UMBZ,KSAY,KSAZ,KSBY,KSBZ,YSAY,YSAZ,YSBY,YSBZ,YOAY,YOAZ,YOB
40 -    plot(T,C(:,1),'-r.',T,C(:,2),'-g.',T,C(:,3),'-b.',T,C(:,4),'-y.',T,C(:,5),'-k.',T,C(:,6),'-m.',T,C(:,7),'-c.')
41 -    title(['Yield Constant for Inhibitor- ALPHA = ',num2str(ALPHA),'  kg/kg']);
42 -    xlabel('Time, h','fontsize',12,'fontweight','b');
43 -    ylabel('Concentrations XA,XB,I,EAZ,EBY,SY,SZ, kg/m^3','fontsize',12,'fontweight','b');
44 -    h = legend('XA','XB','I','EAZ','EBY','SY','SZ',0);
45 -    set(h,'fontsize',11);
46
47 -    FA = C(:,1)./(C(:,1) + C(:,2));
48 -    FB = C(:,2)./(C(:,1) + C(:,2));
49
50 -    figure
51
52 -    plot(T,FA,'-rx',T,FB,'-gv')
53 -    title(['Yield Constant for Inhibitor- ALPHA = ',num2str(ALPHA),'  kg/kg']);
54 -    xlabel('Time, h','fontsize',12,'fontweight','b');
55 -    ylabel('Cell Mass Fractions, FA, FB','fontsize',12,'fontweight','b');
56 -    h = legend('Oxytoca Cell Mass Fraction FA','Aeruginosa Cell Mass Fraction FB',0);
57 -    set(h,'fontsize',11);
58 -    set(title,'fontsize',12,'fontweight','b');
```

Nomenclature

Symbols

CER	Carbon dioxide evolution rate	kg/(m^3 h)
DOT	Dissolved oxygen tension	%
F	Cell mass fractions	-
I	Inhibitor concentration	kg/m^3
K	Saturation and inhibitions constants	kg/m^3
$K_L aC_O^*$	Oxygen transfer rate	kg/(m^3 h)
OUR	Oxygen uptake rate (normalized)	kg/(m^3 h)
S	Substrate concentration	kg/m^3
X	Biomass concentration	kg/m^3
Y	Yield coefficients	kg/kg
E	Level of key enzyme	-
M	Specific maintenance rates	kg/(kg h)
α	Yield constant for inhibitor	kg/kg
β	Consumption rate constant for inhibitor	kg/(kg h)
μ	Specific growth rate	1/h

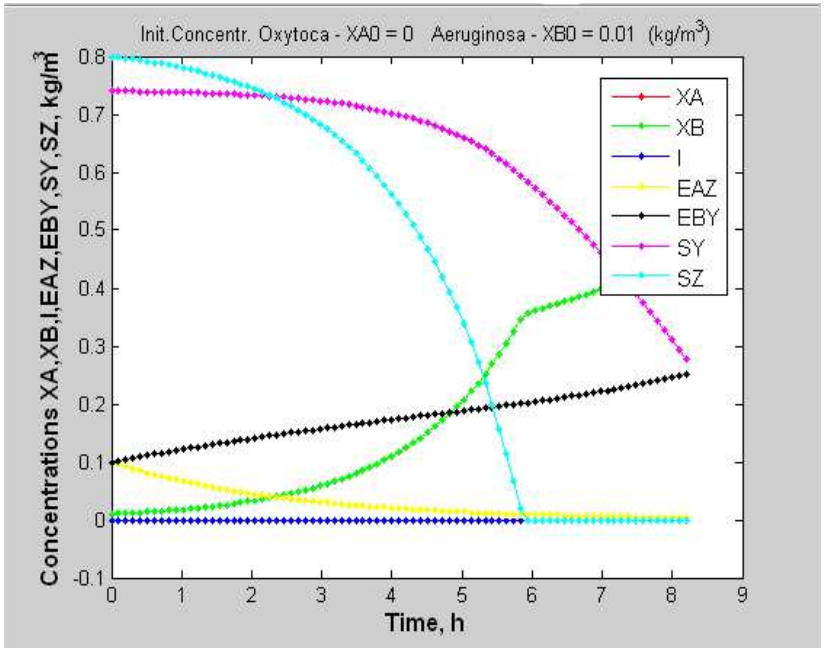
Nomenclature

Indices

A	Refer to *K. oxytoca*
B	Refer to *P. aeruginosa*
C	Refer to carbon dioxide
I	Refer to inhibitor
M	Refer to maximum
O	Refer to oxygen
P	Refer to dilution due to cell division
R	Refer to repression
S	Refer to substrate
Y	Refer to glucose
Z	Refer to citrate

Exercises

1) First test the model for each species separately. Run the simulation with the initial concentration of one species equal to zero. Graph the cell concentration , the substrate concentrations and the key enzyme levels for growth on the secondary substrate against time for the two runs. Notice the substrate preferences for each organism and the differences in key enzyme production.



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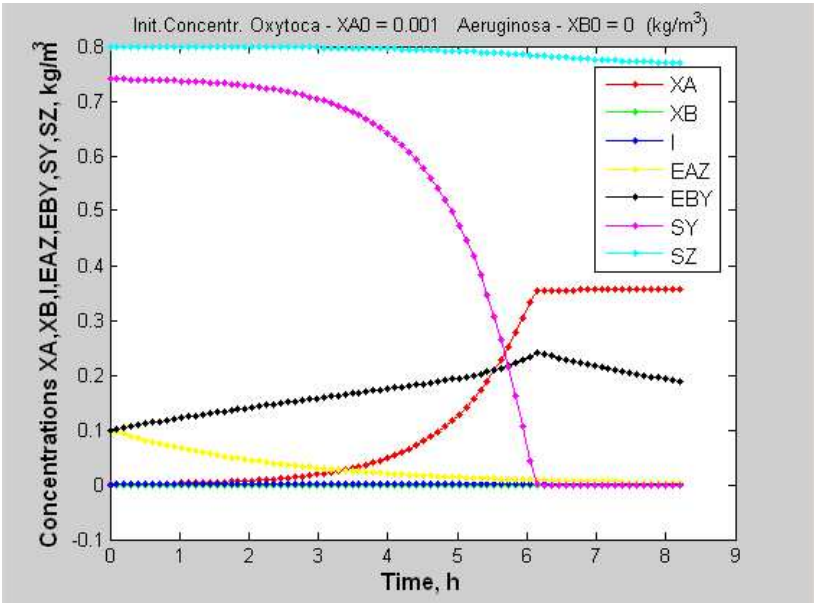
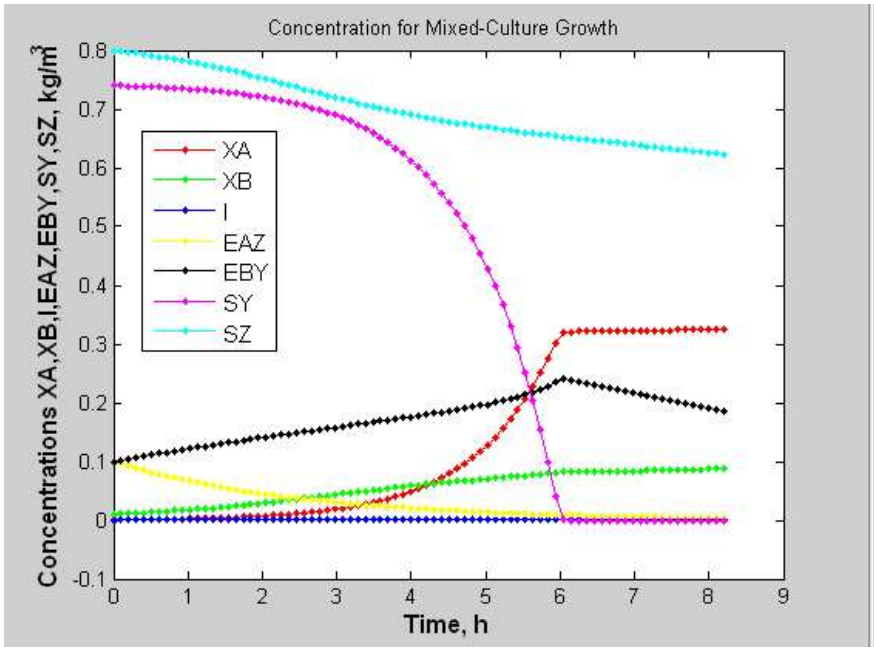
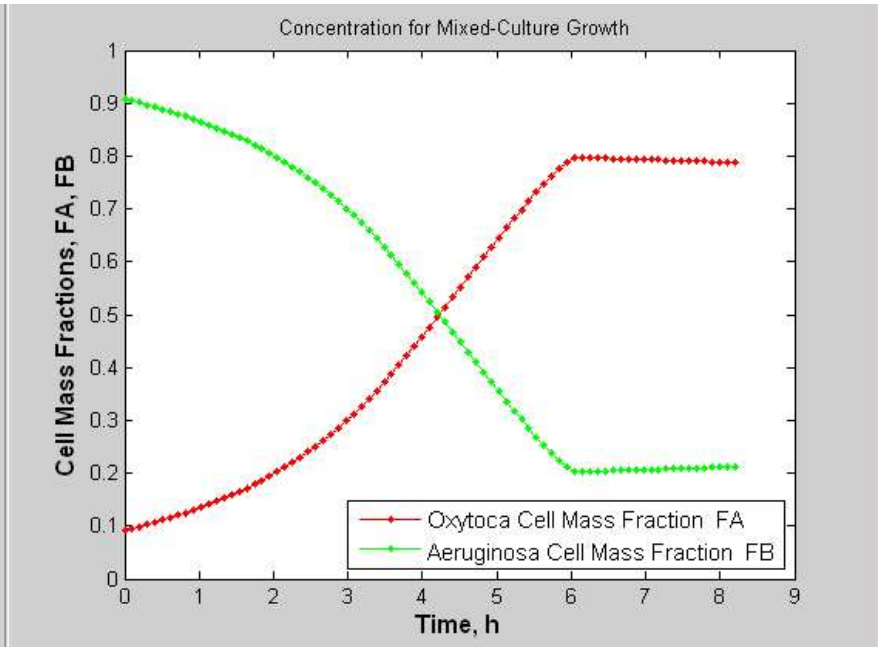


Exercises

2) Run the simulation for mixed-culture growth. Graph the parameters as above. What differences do you see in the production of the two key enzymes? Graph the cell fraction versus time; what are the final levels? What does this tell you about the possibilities of continuous culture under these conditions?





Exercises

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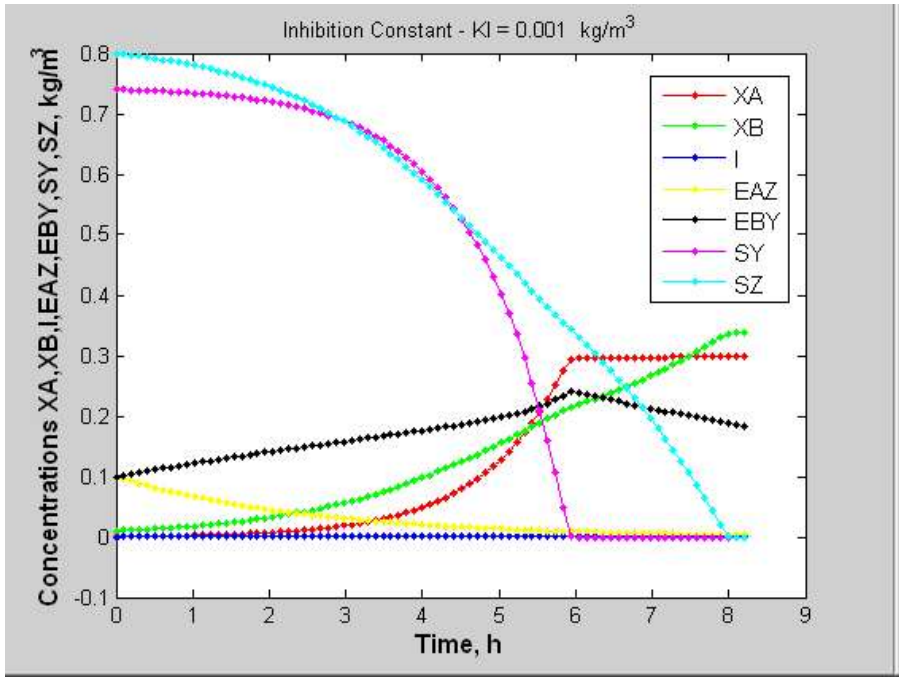

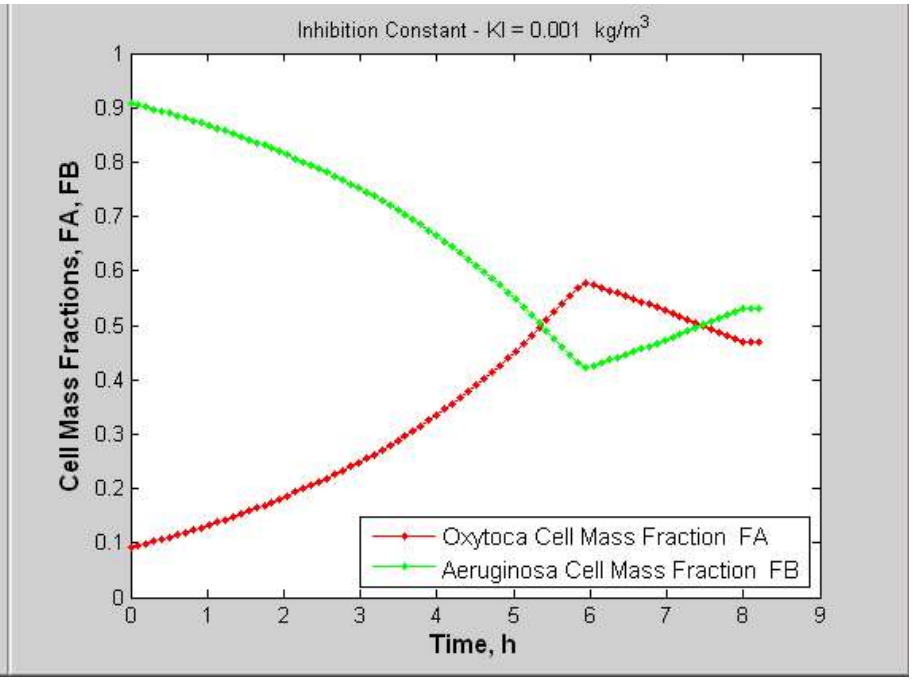

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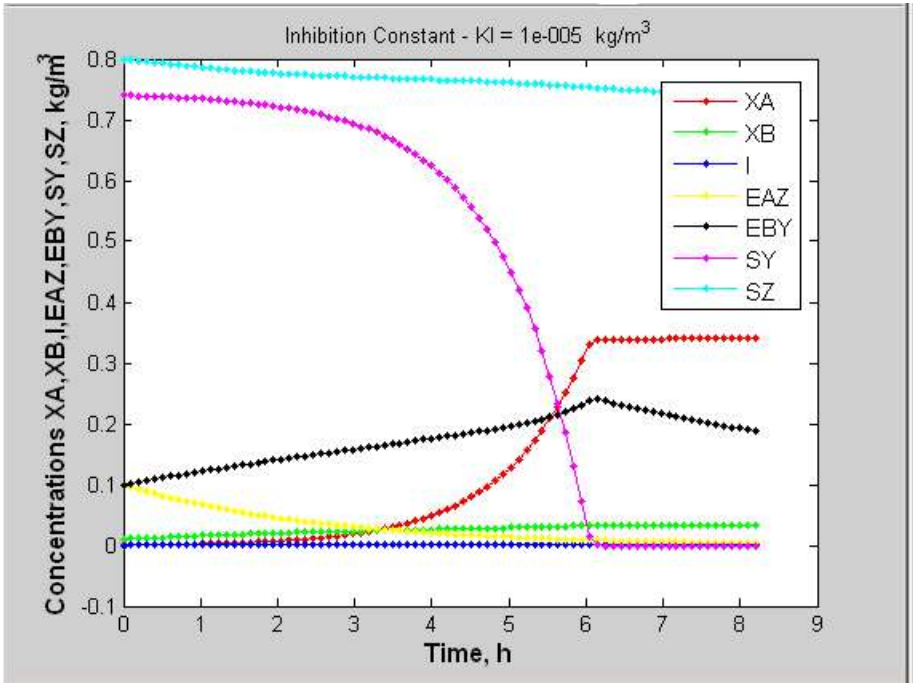

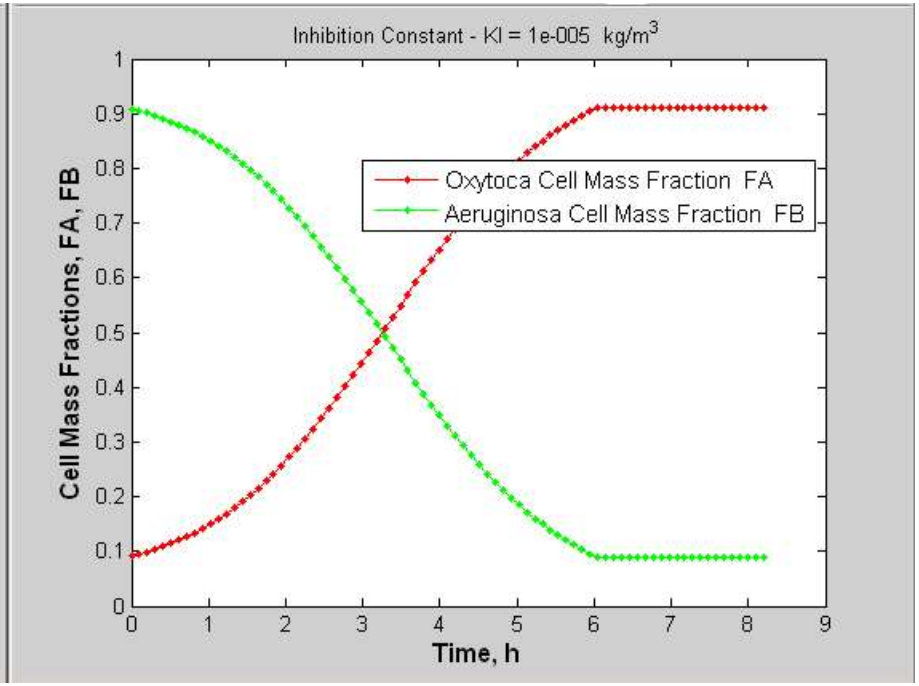

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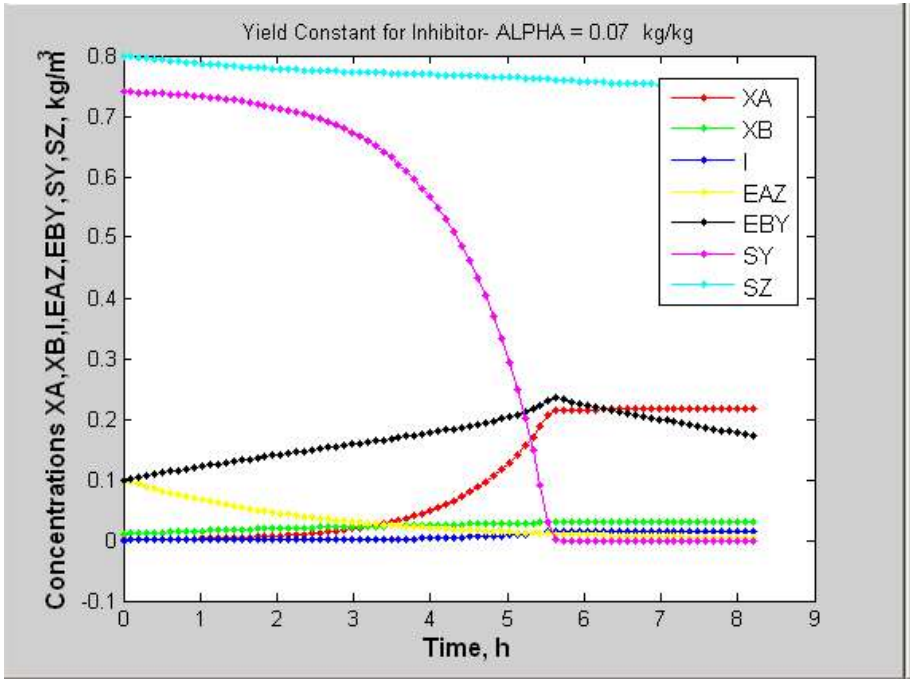
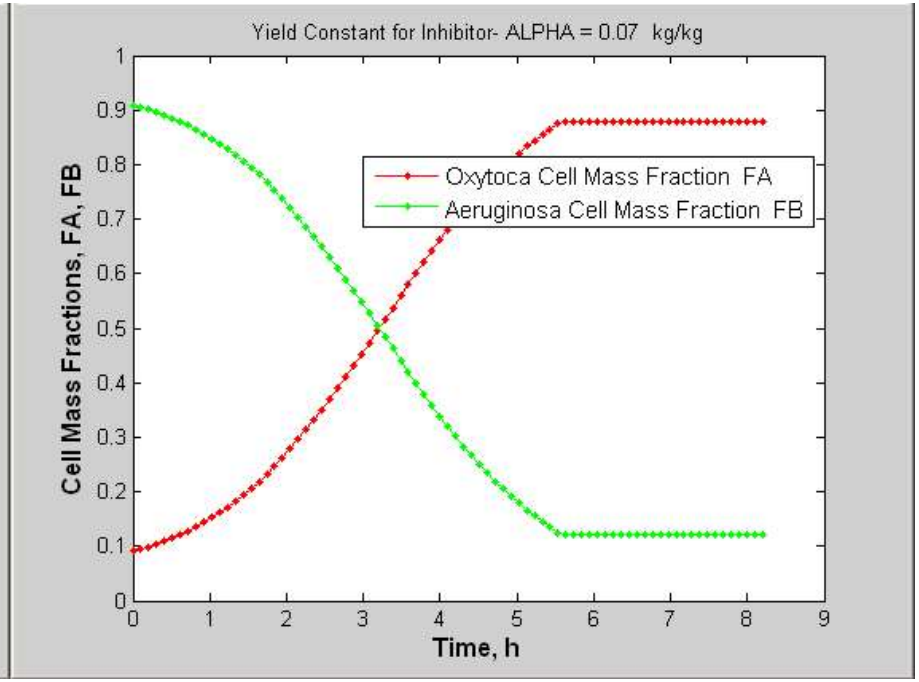
4) Vary α between 0.0007 and 0.07. What happens to the cell fractions and the rate of inhibitor production? What effect does this have on key enzyme level production?





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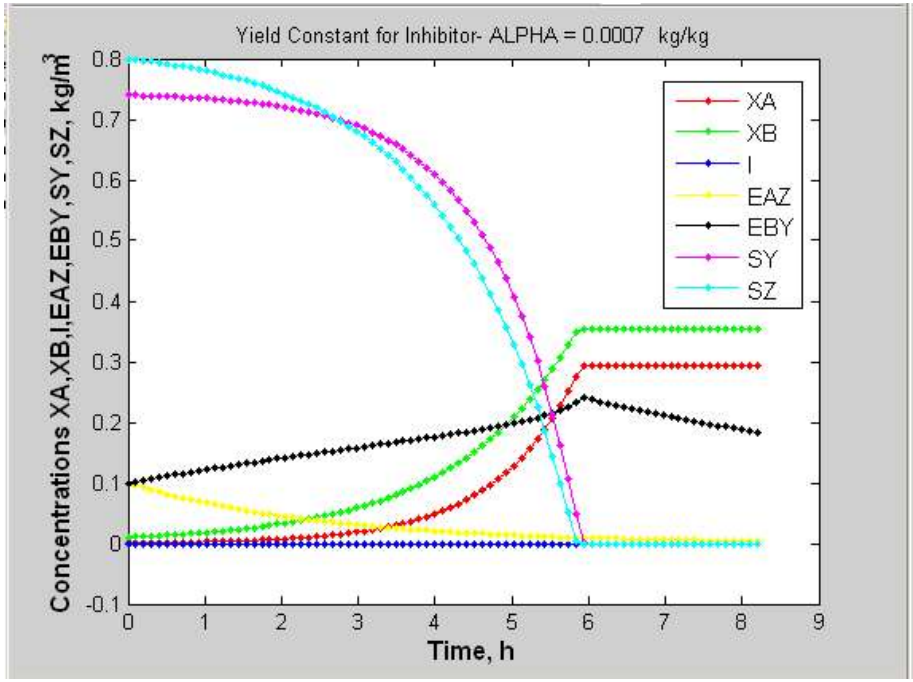
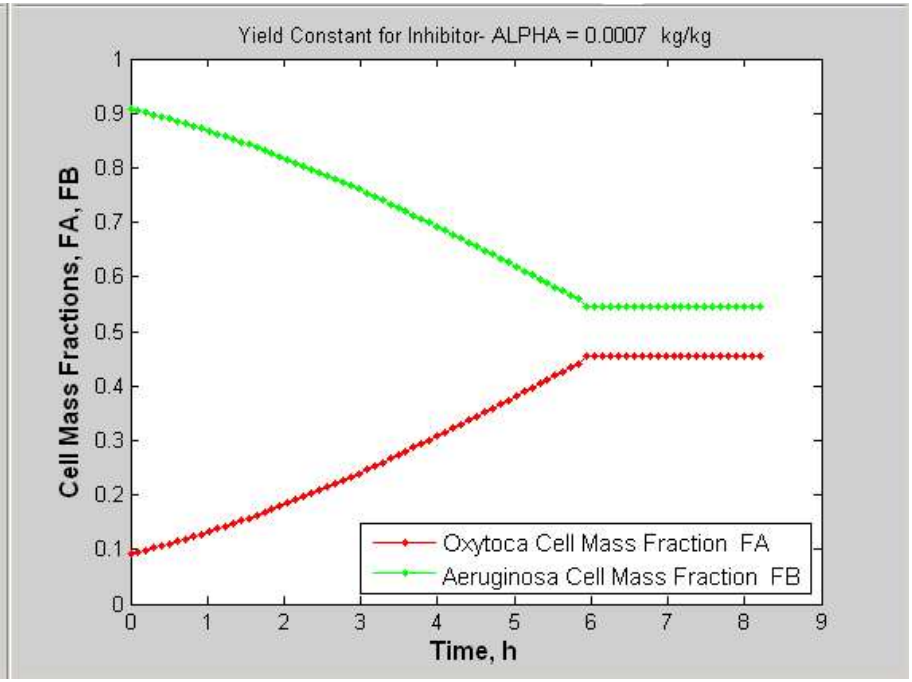
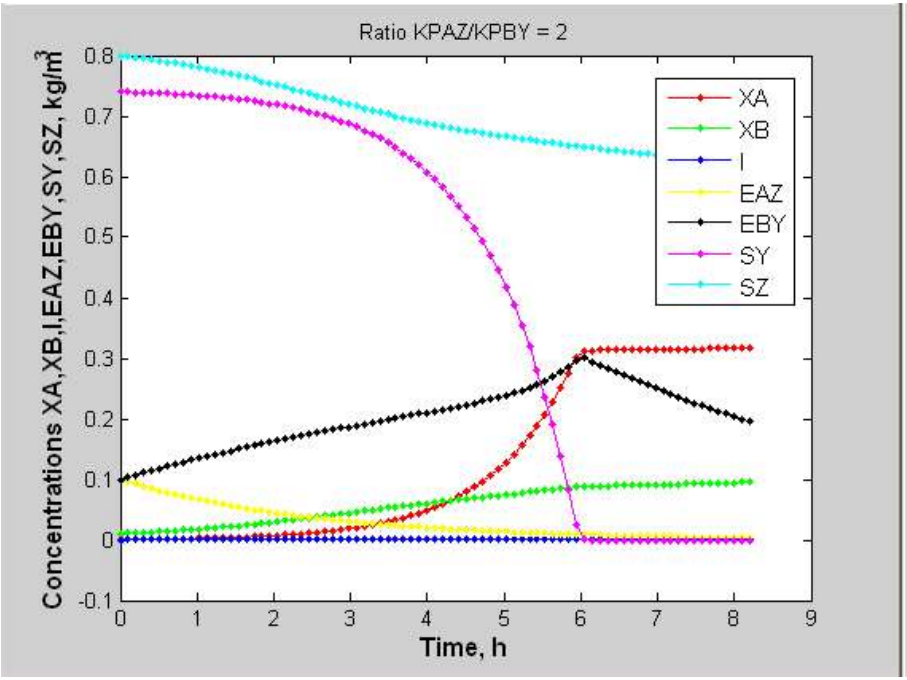
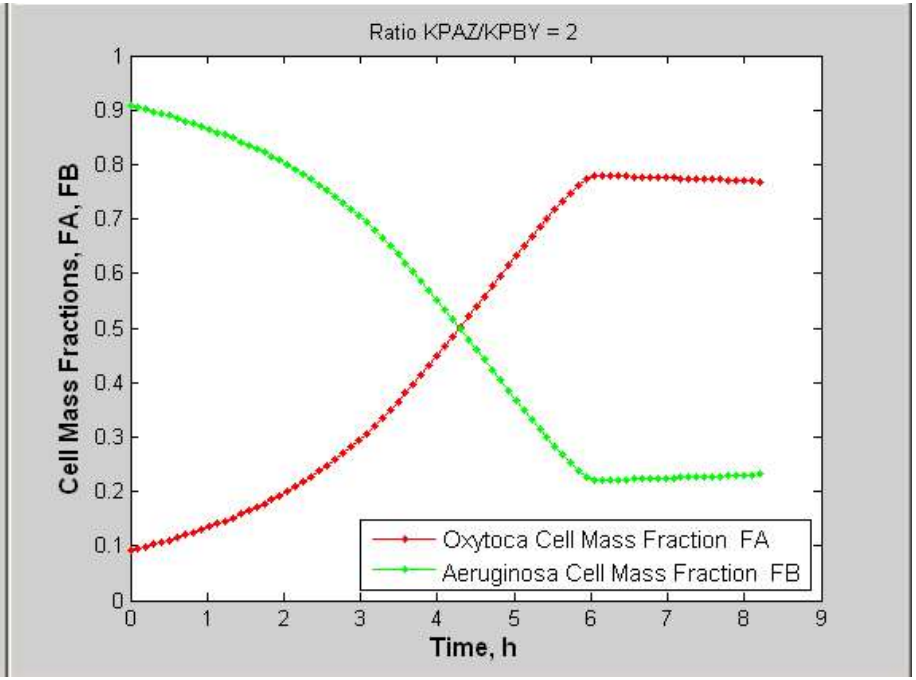




Exercises

5) Vary the ratio k_{PAZ} /k_{PBY}. What effect does this have on the time necessary to exhaust citrate?





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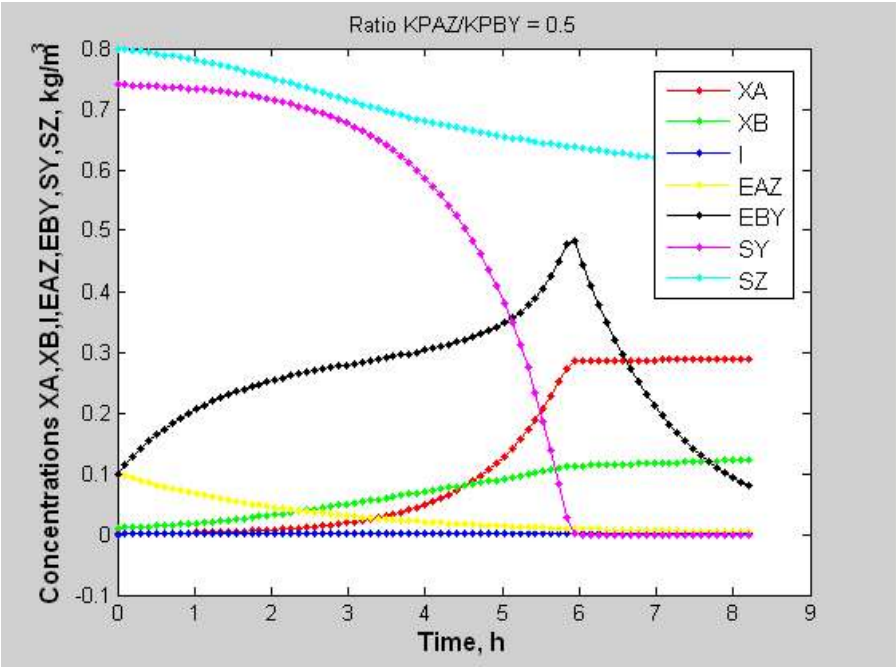

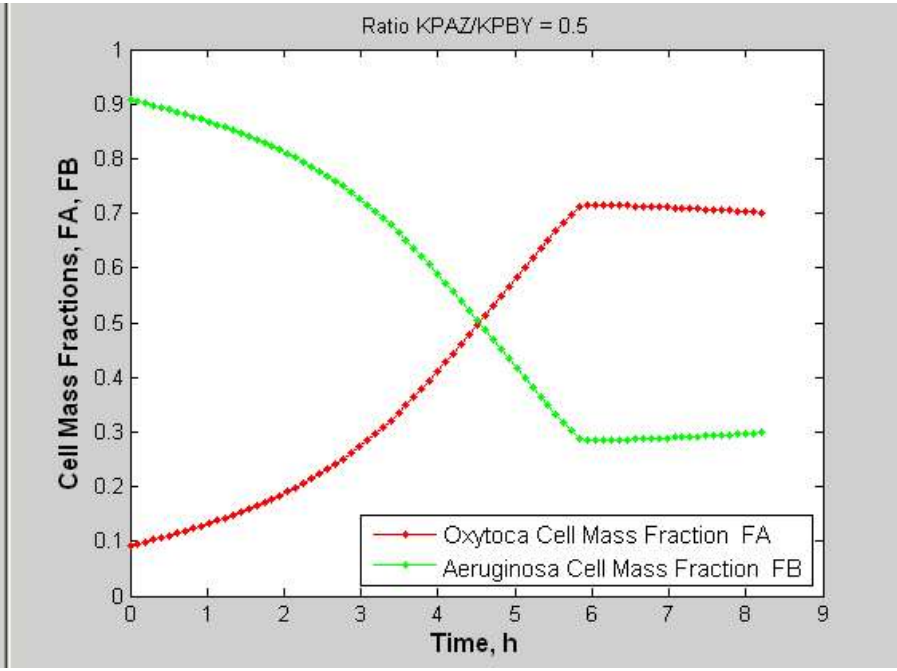

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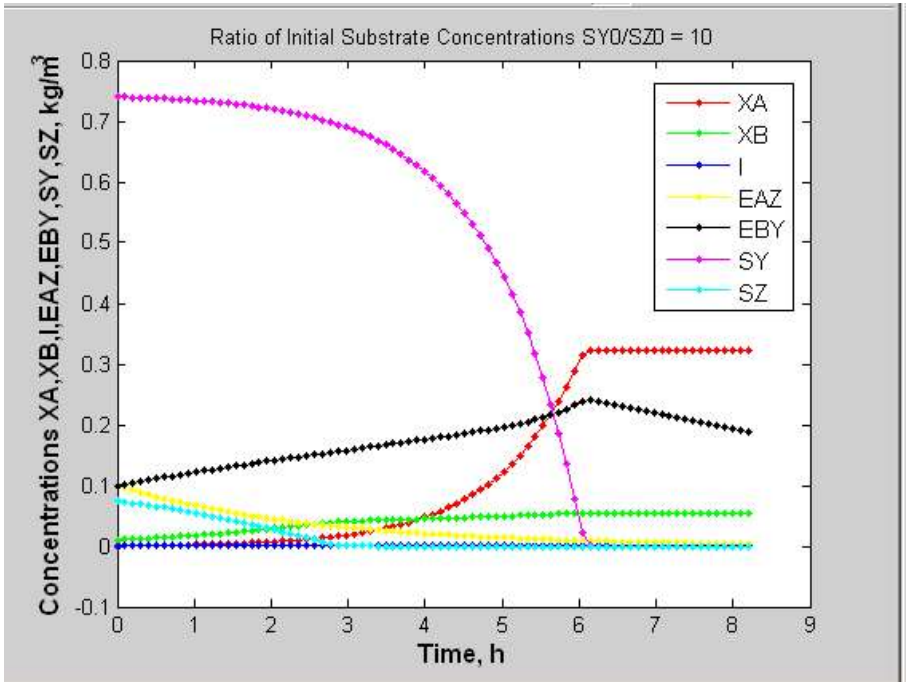
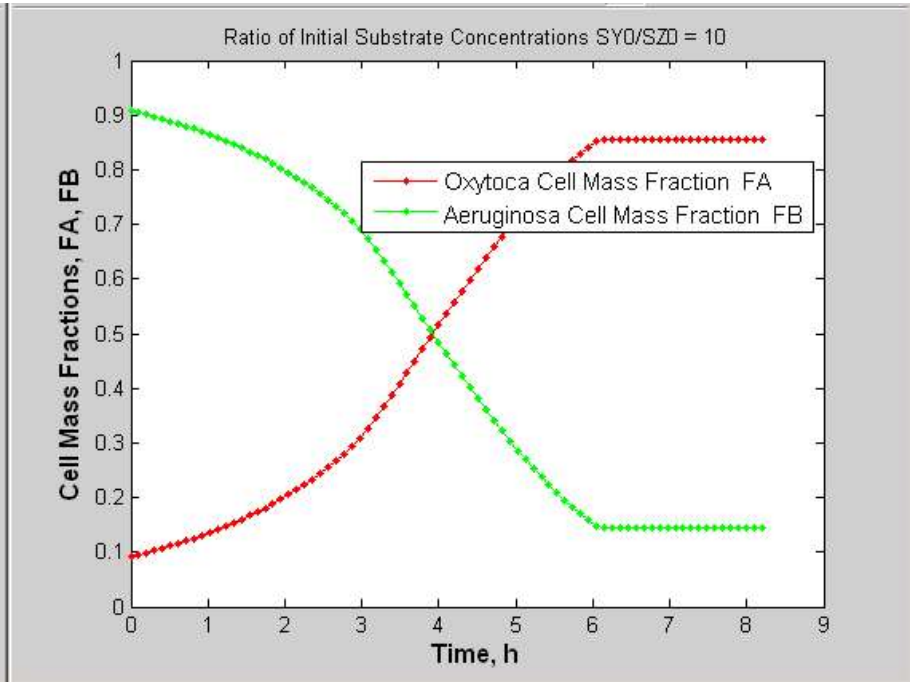
6) Vary the ratio of initial concentration of substrates from 0.1 to 10. At which values do the changes between runs become evident? Why?





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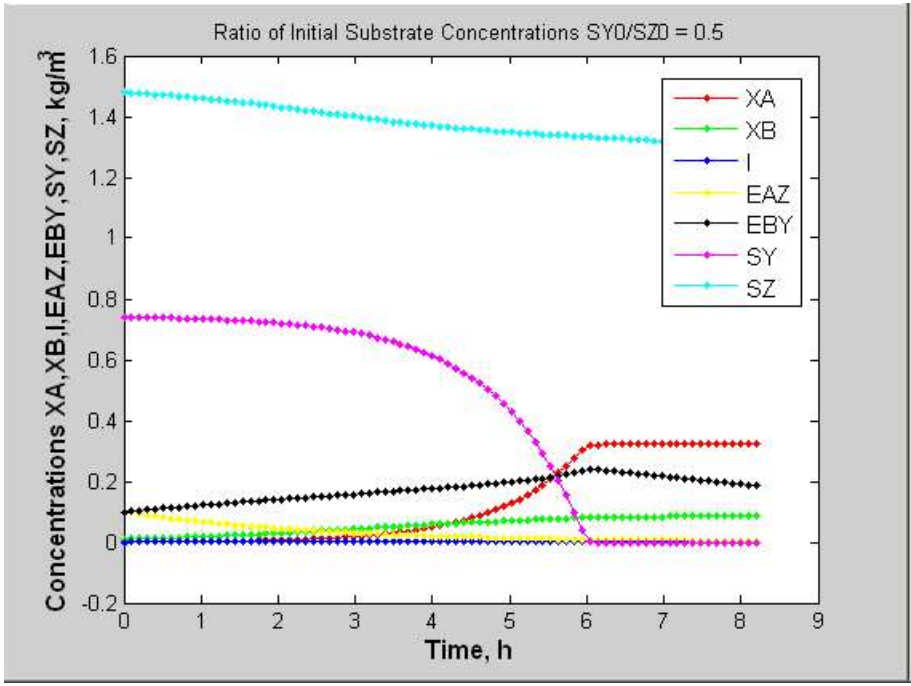

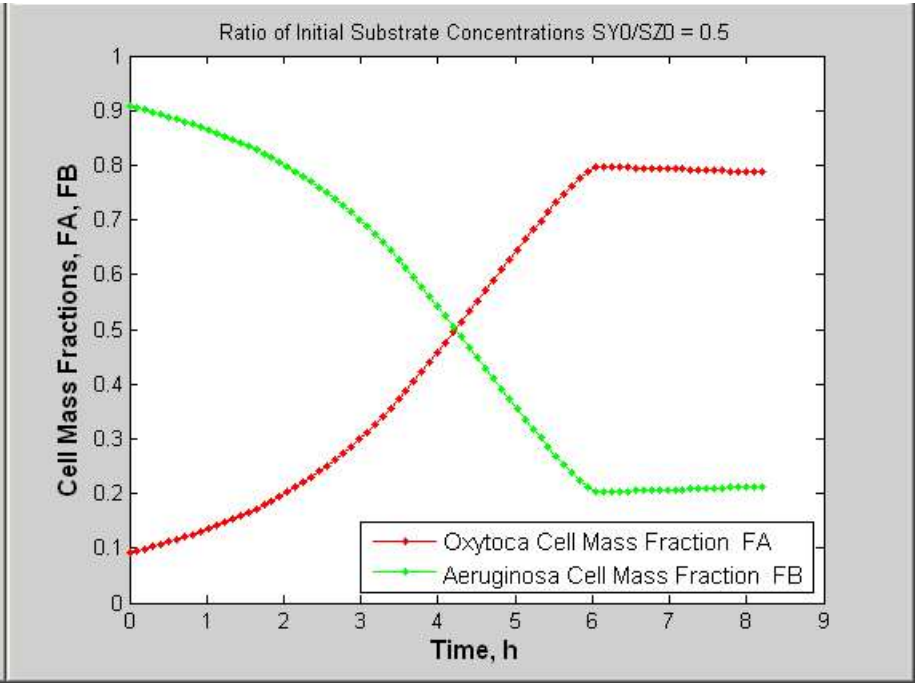

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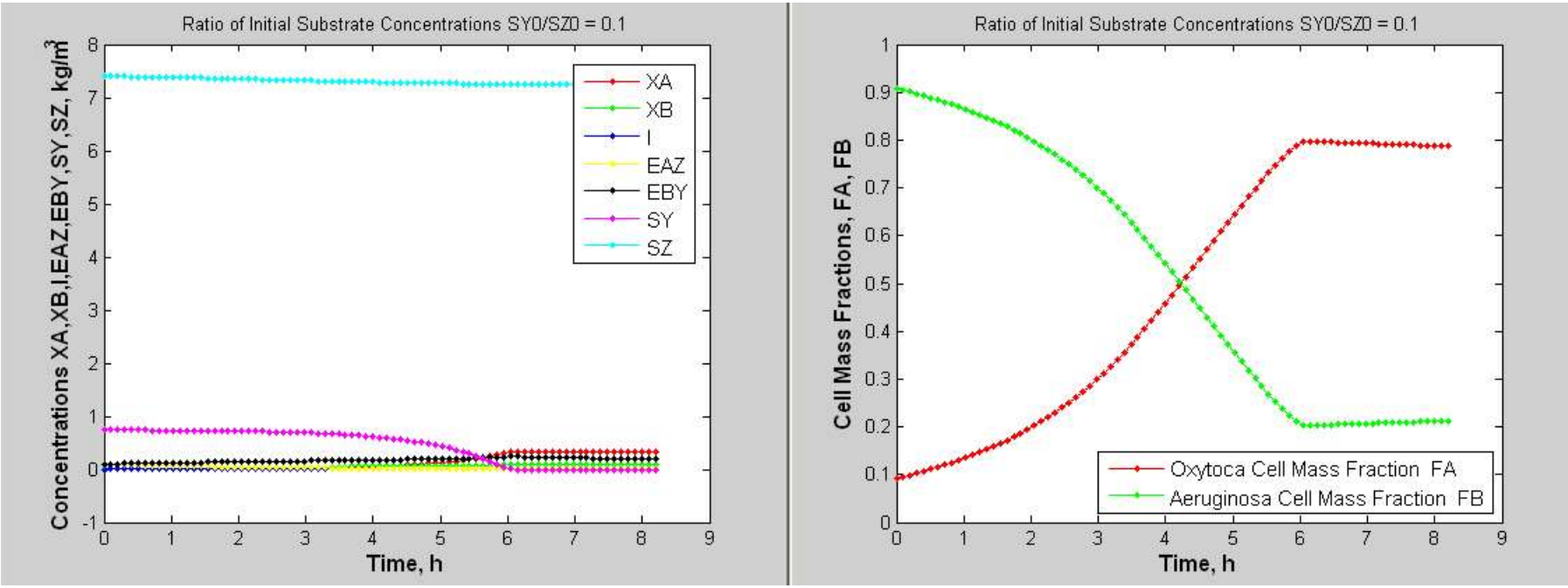

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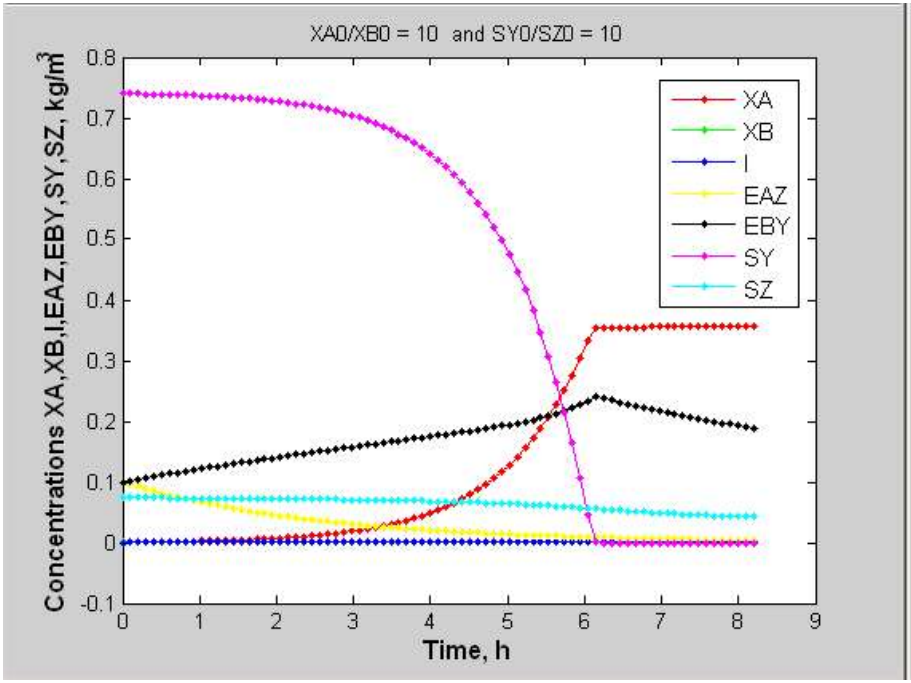

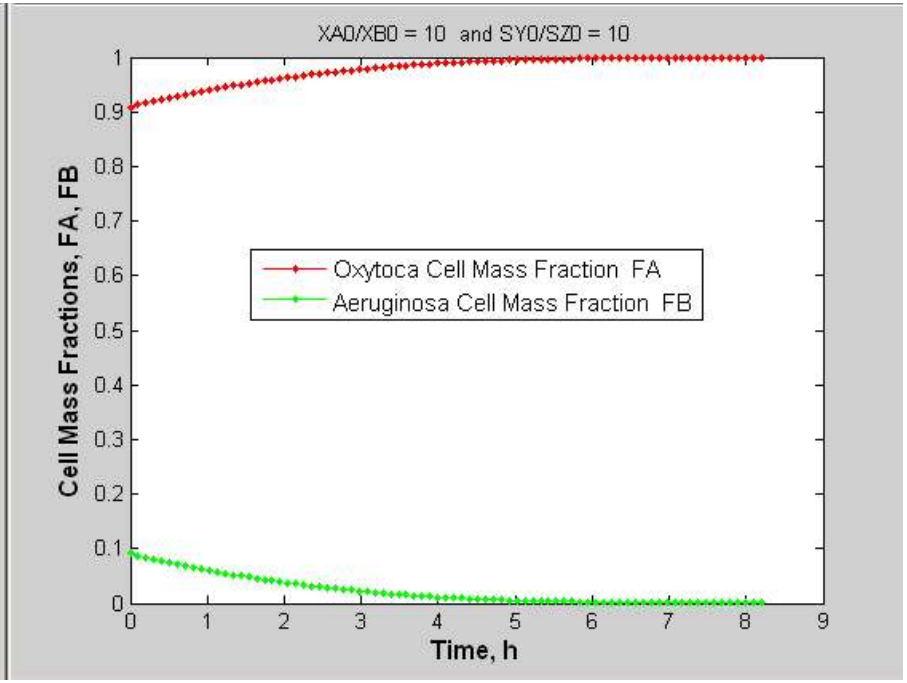

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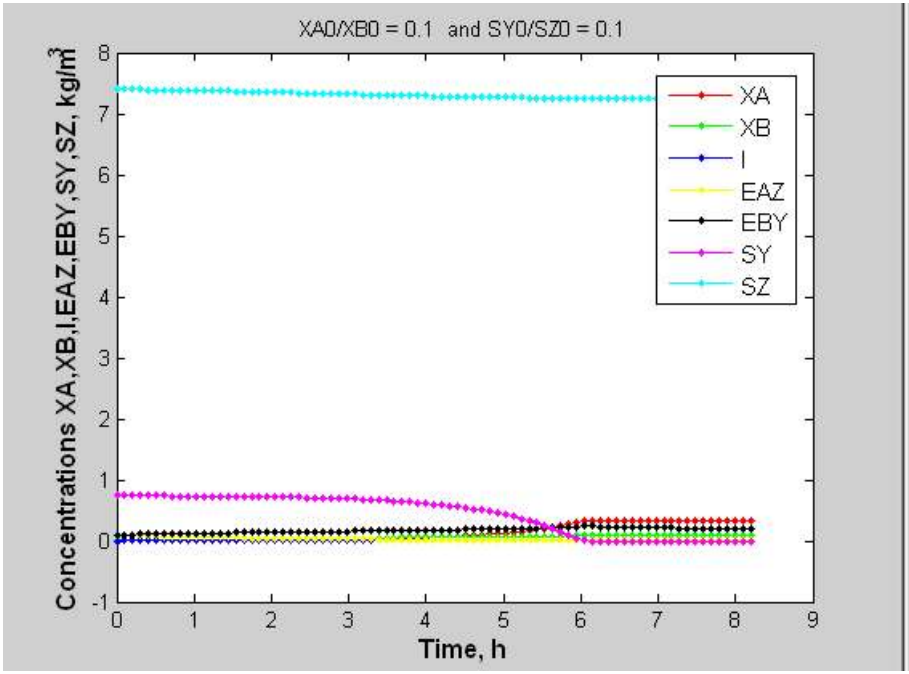

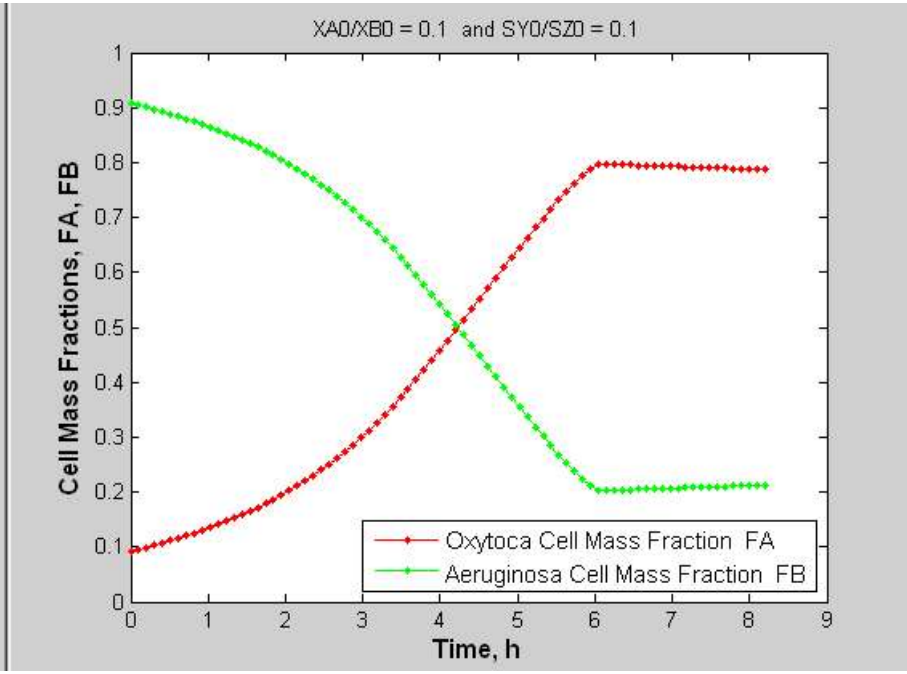

Exercises

8) Alter the model for continuous culture.