Modeling the Fermentation of Beer with Brewer's Yeast

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Introduction

It is common knowledge that alcohol is an integral part of society today. The United States is the second largest producer of beer globally, responsible for producing over 190 million barrels of beer per year.[1, 2] The fermentation process begins with the grains to be used as a source of sugar. There are many kinds of beer that can be produced: dark, light, etc. In order to produce these different beers, one must consider the types of grains used, the strains of yeast, and when to halt the fermentation process. Most beers use combinations of numerous grains, including hops, barley, and rye as the big three. The type of grains used impacts the sugars available. Different yeasts will convert the sugars to different products or ratios of those products. Yeasts can be top fermenting or bottom fermenting, based on where the cells aggregate during the fermentation process. Dark lagers are produced using bottom-fermenting strains while light beers are produced with top-fermenting strains. The strains of yeast used also impact the fermentation process. Most industrial brewers use upwards of 600 strains of yeast and vary the temperature over the course of the fermentation to adjust the yeasts' activity. Temperature is very important for the fermentation process; too cold and fermentation will not occur, too hot and the cells will die. Typical fermentation times range from 3-4 days upwards of 4 weeks, depending on the desired beverage to be produced.

People who wish to ferment their own beer at home can do so by simply buying their own yeast. Brewer's yeast is typically going to be the mix of choice, containing all of the different strains necessary for fermentation. Most of the yeast cells in a fresh packet of yeast will be dormant, or in a lag phase, while a small fraction will be active. On average, 15% of the initial amount of yeast will die every month that it has been sitting.[3] Feed is also a factor to be considered. The feed can be purchased pre-mixed or one can use table sugar. Table sugar is typically not used for fermenting beer, but it is quite popular for producing moonshine. For the in-home fermentation, the reaction will take place at room temperature. Optimum temperatures for these yeast strains occur right around room temperature, which is convenient for the fermentation process because a lot of energy goes into manipulating temperature to regulate the production of ethanol.

The purpose of this project is to model the population growth, production of ethanol, depletion of sugar substrate, and to determine an optimal time to harvest the beer. This has an intended application for home-brewers with smaller batches and fewer regulatory capabilities.

Methods

In order to do the proposed modeling, several assumptions must be made. The first of which is that the temperature is held constant at room temperature. Heat is a by-product of fermentation, and this could raise the temperature of the system. However, it is unlikely that the heat produced is enough to significantly alter the temperature of the system due to the high heat capacity of the water. Another critical assumption is that the yeast cells haven't

been sitting for an extended period of time, attributing to a large percentage being dead. Most of the cells will be dormant, assumed to be about 95% of the inoculated cells, and the rest will be active.

Most of the equations used to make the calculations are first order differential equations that are all interdependent upon each other. The Euler method should be a good approximation of the analytical solution. Many of the parameters are temperature dependent and were fitted using the Arrhenius equation (equation 1). The kinetic models and values for the Arrhenius equations were taken from de Andres-Toro.[4] Table 1 shows all of the values determined for the Arrhenius equation.

$$\mu_{i_0} = e^{(A_i + {B_i}/{T})} \qquad (1)$$

The equations used to calculate the concentrations of cells, sugar, and ethanol are listed below in equations 2, 3, and 4. The equation for the population growth can be interpreted as the cell population growing from the replication of the active cells (term 1) and the transition of dormant cells into the active phase (term 3). The death term (term 2) is the only presumed reason for a loss in population. This equation is only valid for when the cells are not in the lag phase, determined to be when at least 80% of the cells inoculated are active. Until that time, the cell population, and the concentrations of sugar and ethanol, are determined by equation 5. Most packages of yeast that are purchased provide how many cells are in a given unit. To determine the optimal time to stop the fermentation, it was arbitrarily chosen that once 95% of the anticipated ethanol saturation level is reached, the fermentation should stop. This was decided because the rate of ethanol production decreases tremendously and it takes a decent amount of time after this point to reach full saturation. The methodology used to achieve this was by fitting the final few points (linearly) of the ethanol concentration

curve and using the y-intercept (an extrapolation technique) to determine the maximum saturation level. Then a loop was implemented to find the point on the ethanol concentration curve that was within a certain tolerance of 95% of the y-intercept.

$$\frac{dX_{act}}{dt} = \mu_x(T,t) \cdot X_{act}(t) - \mu_{DT}(T) \cdot X_{act}(t) + \mu_L \cdot X_{lag}(t) \quad t > t_{lag} \qquad (2)$$

$$\frac{dC_s(t)}{dt} = -\mu_S(T,t) \cdot X_{act}(t) \qquad (3)$$

$$\frac{dC_e(t)}{dt} = f(t) \cdot \mu_e(T,t) \cdot X_{act}(t) \qquad (4)$$

$$\frac{dX_{act}(t)}{dt} = \mu_{lag} \cdot X_{lag}(t) \quad t < t_{lag} \qquad (5)$$

The terms in the above equations can be calculated as follows in equations 6-9. These equations are the specific growth rates, except for equation 9. Equation 9 is an inhibition factor that accounts for the decreasing production rate of ethanol over time.

$$\mu_x = \frac{\mu_{x_0} \cdot C_s(t)}{k_x + C_e(t)} \qquad (6)$$

$$\mu_s = \frac{\mu_{s_0} \cdot C_s(t)}{k_s + C_s(t)} \qquad (7)$$

$$\mu_e = \frac{\mu_{e_0} \cdot C_s(t)}{k_e + C_s(t)} \qquad (8)$$

$$f = 1 - \frac{C_e(t)}{0.5 \cdot C_{s_0}} \qquad (9)$$

To take things further, probabilities can be taken into account. These functions were taken from Subramanian's work on population modeling.[5] The gamma function (equation 10) considers the probability of a cell of mass m replicating. The function $r(m, C_s)$ seen in equations 10-13 is a growth rate of change of a single cell's mass. Equation 14 calculates the probability of the daughter cell having a particular mass, m, if the parent cell has a certain mass, m'. The code allows the user to input a desired mass of the parent cell to see the most probable daughter cell mass. Most yeast cells have masses on the order of picograms. The function W represents the number of cells in solution having a certain mass. There is no analytical solution to the general function of W at any time, t, however a function for t = 0 was determined by Subramanian. Table 2 lists the values of all the constants seen in all equations.

$$\Gamma(m, C_s) = \frac{2}{\varepsilon\sqrt{\pi}} \cdot \frac{e^{-\left(\frac{m-m_c}{\varepsilon}\right)^2}}{erfc(\frac{m-m_c}{\varepsilon})} \cdot r(m, C_s) \qquad (10)$$

$$r(m, C_s) = r'(m, C_s) - r''(m, C_s) \qquad (11)$$

$$r'(m, C_s) = \frac{2}{R\rho} \cdot \frac{\varphi C_s m}{k_s + C_s} \qquad (12)$$

$$r''(m, C_s) = \mu_c \cdot m \qquad (13)$$

$$p(m, m') = \frac{30m^2(m'-m)^2}{m'^5} \qquad (14)$$

$$W(m, 0) = \frac{m}{\varepsilon} e^{-m/\varepsilon} * 10^{24} \qquad (15)$$

Parameter	Description	A_i	B_i
μ_{x_0}	maximum cell growth rate	108.31	-31934.09

μ_{s_0}	maximum sugar consumption	-41.92	11654.64
μ_{e_0}	maximum ethanol production rate	3.27	-12667.24
μ_{DT}	specific cell death rate	130.16	-38313
μ_L	specific cell activation rate	30.72	-9501.54
k_e	affinity constant	-119.63	34203.95
k_s	affinity constant	-119.63	34203.95
k_x	affinity constant	-119.63	34203.95

Table 1: Values for the Arrhenius equation were calculated in previous experiments by fitting them over a range of temperatures. They are all dependent on temperature only (Kelvin).

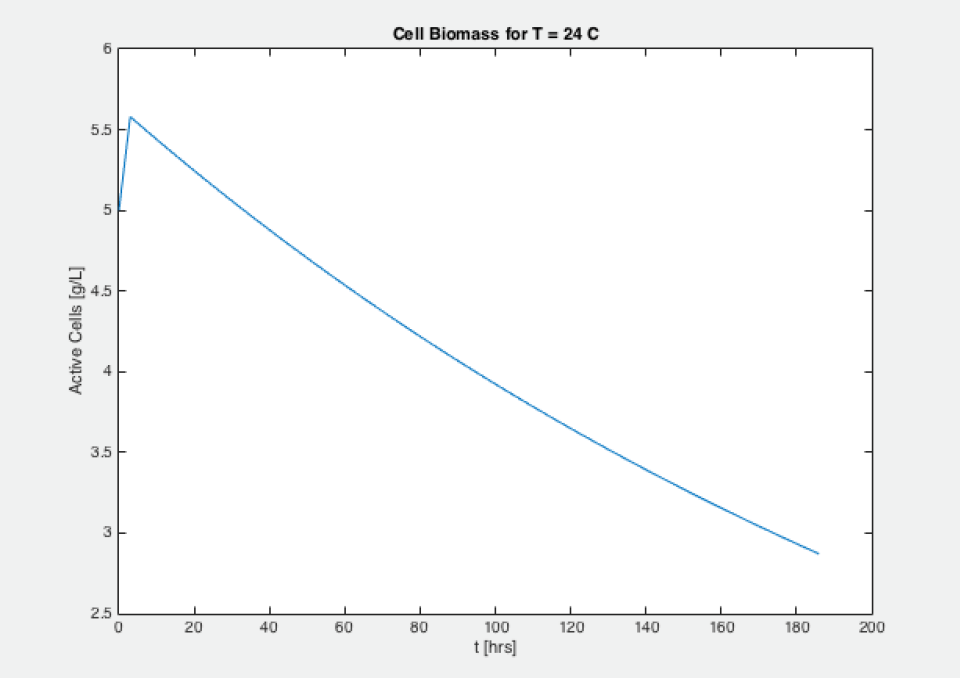
Constant	Description	Value
m_c	Mean cell mass (g)	$3 \cdot 10^{-12}$
ε	Measure of the spread of division mass about mean mass (g)	$3\sqrt{2} \cdot 10^{-13}$
ϕ	Maximum influx of mass across cellular membrane (g/cm^2·hr)	$6 \cdot 10^{-5}$
μ_c	Specific rate of mass released by the cells (hr^{-1})	1
ρ	Average density of a cell (g/cm^3)	1.01
R	Radius of cell (m)	$5 \cdot 10^{-6}$
k_s	Michaelis constant (g/L)	0.02

Table 2: A list of constants used.

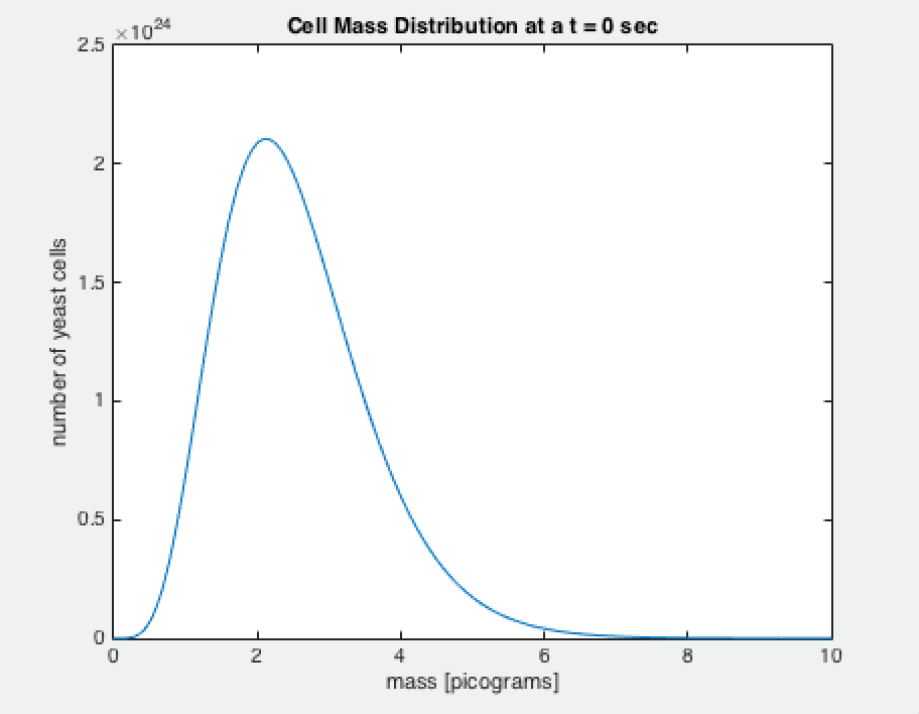
Results and Discussion

The cell biomass plot (Graph 1) suggested the expected trends, of growth to a maximum followed by death. The death is linked to the depleted sugar concentration. Also, growth is inhibited by the concentration of ethanol, however this doesn't impact it much. The numerical values do not appear to be incredibly accurate, but are "proportional" to what is expected. Graph 2 shows the initial cell mass distribution at t = 0. The average cell mass will

be slightly higher than the mass corresponding to the peak. For yeast, the average cell mass is about $3 \cdot 10^{-12}$ grams.

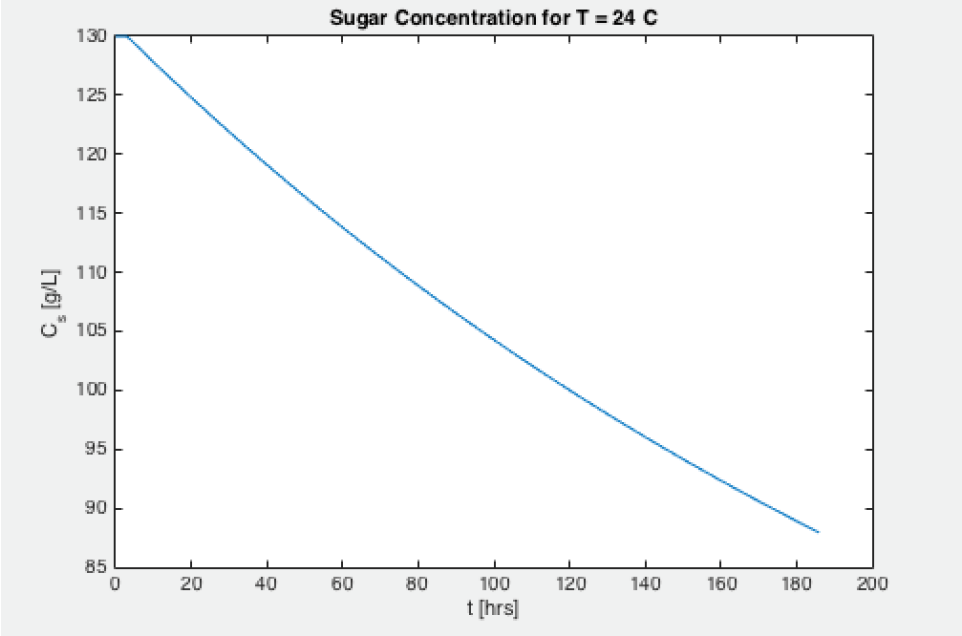


Graph 1: The cell biomass should start off with a delay due to the estimated fraction of inoculated cells in the lag phase. However, this isn't always the case and the results for the given starting conditions are possible.

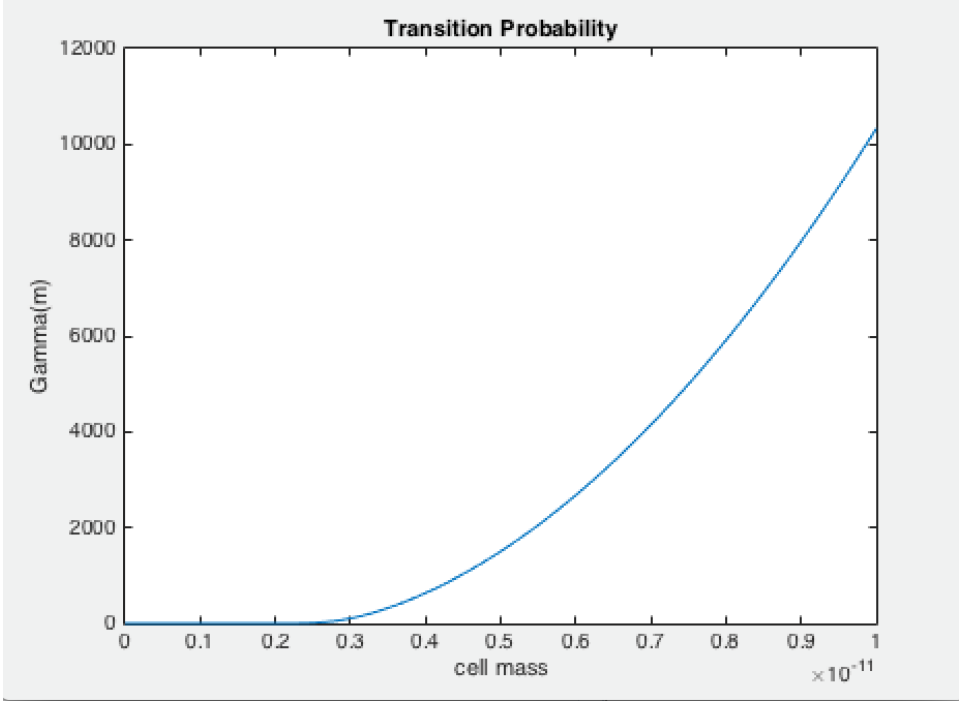


Graph 2: This graph shows the initial cell mass distribution at t=0. It does not appear to be symmetric about a certain mass.

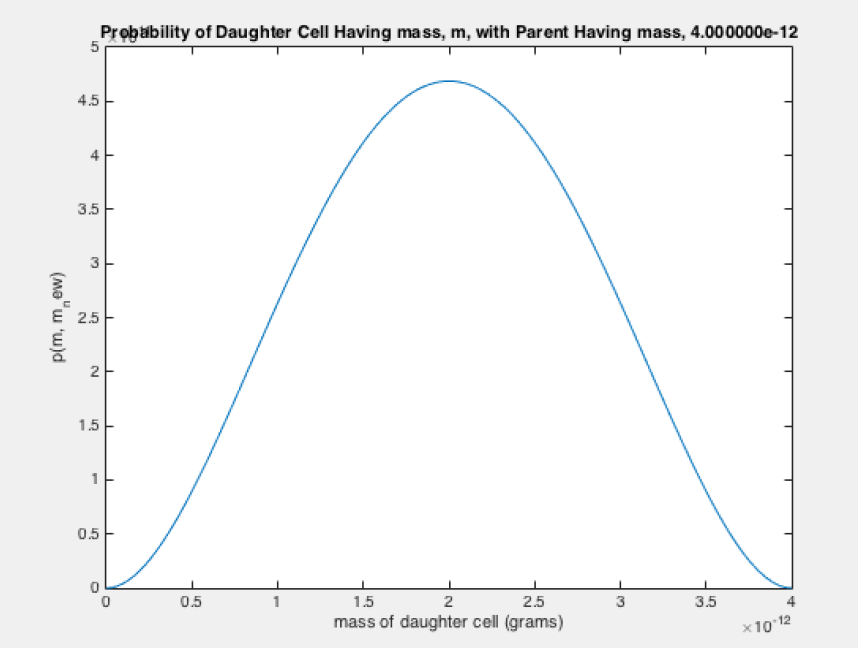
The sugar substrate concentration (Graph 3) also appears to be good and reflect decent values. There is a short lag before the concentration begins to fall. This is normal because of the lag phase. As the cells begin to grow and consume more, they will replicate, leading to an increase in consumption. The probabilities of a cell splitting (Graph 4) once a certain mass is attained appear to be very accurate. The graph can be interpreted as the heavier the cell, the more likely it is to split than a less massive one. Graph 5 shows an example probability chart for a range of possible daughter cell masses. The input was 4 picograms, and the most probable mass was half of that, 2 picograms. It makes sense that a cell would split equally into two cells.



Graph 3: This is the anticipated sugar concentration function over the course of the fermentation. There is a lag before it begins to decrease, which can be attributed to the delay before the yeast cells become active.

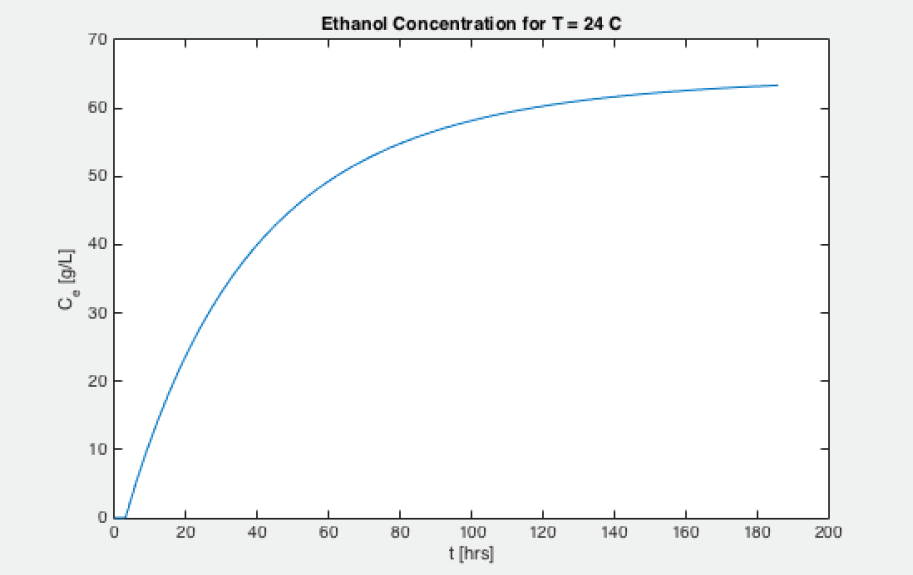


Graph 4: The transition probability is indicative of how likely a cell is to divide based on its mass.



Graph 5: The distribution of potential daughter cell masses is symmetric. It is most likely that a parent cell will divide evenly in halves.

The production of ethanol (Graph 6) followed the expected curve line. When certain values were inputted that matched experimental data, the final ethanol concentration was close to literature values.[6] The soonest it reaches the maximum concentration value is at 96 hours. So, the estimation saves 15 hours. For the conditions inputted, the program predicts that 95% of the maximum ethanol concentration occurs at 81 hours (almost 3.5 days). Looking at Graph 6, it can be seen that there isn't much change in the ethanol concentration over a substantial period of time.



Graph 6: The ethanol concentration is as expected, starting at zero and then increasing to a maximum value.

All of the functions are interdependent, but the cell concentration of particular importance because it directly determines the sugar and ethanol concentrations. If this is incorrect, there can be significant errors that arise. Cell population models are oscillatory in nature, raising concerns about the validity of even using the Euler method for this application. Alternative algorithms include the Runge-Kutta method, which is reputable for its applications to oscillatory motion. For future work, it would be beneficial to explore alternative algorithms to solve the differential equations.

References

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3. Yeast Starters for Home Brewing Beer – Part 1 | Home ... Yeast Starters for Home Brewing Beer – Part 1 | Home ..., http://beersmith.com/blog/2010/12/14/yeast-starters-for-home-brewing-beer-part-1/ (accessed May 12, 2016).

4. Andrés-Toro, B. D.; Girón-Sierra, J.; López-Orozco, J.; Fernández-Conde, C.; Peinado, J.; García-Ochoa F. A Kinetic Model for Beer Production under Industrial Operational Conditions. *Mathematics and Computers in Simulation*.1998, *48*, 65–74.

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Appendix

```
function Brigham_Fermentation(N, S0, V)

%function Brigham_Fermentation(N, S0, V)
%
%Background:
%This function considers the number of Brewers yeast cells (mostly S.
%cervisiae inoculated into a Beer fermenting system. The function will
%output the evolution of the yeast population, the consumption of the
%sugar substrate, and the production of ethanol with respect
%to time. Many calculations are made using the Euler approximation for
%interdependent first order differential equations. The optimal time to
%stop the fementation is outputted as an fprintf function.
%
%Assumptions:
%(1)The substrate has been heated up to form the mash (wort) and has cooled down
%   to the desired temperature of fermentation.
%(2)Temperature is held constant at room temperature
%
%Inputs:
%N: number of yeast cells inoculated
%S0: initial sugar substrate concentration (good values are between 80 and 200)
%V: volume in Liters of water used in the fermentation (good values are
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%in the range of 20L {about 5 gallons})


%======================Calculate the starting parameters

T = 24 + 273.15;                          %convert Celsius to Kelvin

mu_x0 = exp(108.31-31934.09/T);           %max cell growth rate
mu_s0 = exp(-41.92+11654.64/T);           %max substrate consumption rate
mu_lag = exp(30.72-9501.54/T);            %specific cell activation rate
mu_DT = exp(130.16-38313/T);              %specific cell death rate
mu_e0 = exp(3.27-1267.24/T);              %max EtOH production rate
k_e = exp(-119.63+34203.95/T);            %affinity constant
k_s = k_e;                                %affinity constant
k_x = k_e;

deltat = 0.01;
t = 0:3:200;                              %time to collect data

C_s = zeros(1, length(t));
C_s(1) = S0;                              %initial concentration of sugar
mu_s = zeros(1, length(t));
mu_s(1) = mu_s0*C_s(1)/(k_s + C_s(1));

C_e = zeros(1, length(t));
C_e(1) = 0;
mu_e = zeros(1, length(t));
mu_e(1) = mu_e0*C_s(1)/(k_e+C_s(1));

X_act = zeros(1, length(t));              %Number of active cells
X_act(1) = 0.05*N;                        %Initial number of active yeast cells

mu_x = zeros(1, length(t));
mu_x(1) = mu_x0*C_s(1)/(k_x+C_e(1));

f = zeros(1, length(t));
f(1) = 1-C_e(1)/(0.5*S0);

X_lag = zeros(1, length(t));
X_lag(1) = 0.95*N;

%======================Calculate the concentrations and rates with Euler Method

for n = 2:length(t)-2
    X_act(n) = X_act(n-1) + deltat.*(X_act(n-1).*(mu_x(n-1)-mu_DT)+
X_lag(n)*mu_lag);
    X_lag(n) = mu_lag*(N-X_act(n));
    C_s(n) = C_s(n-1) - deltat.*(mu_s(n-1).*X_act(n-1));
    C_e(n) = C_e(n-1) + deltat.*(f(n-1).*mu_e(n-1).*X_act(n-1));

    mu_x(n) = mu_x0.*C_s(n)./(k_x+C_s(n));
    mu_s(n) = mu_s0.*C_s(n)./(k_s+C_s(n));
    mu_e(n) = mu_e0.*C_s(n)./(k_e+C_s(n));
    f(n) = 1-C_e(n)./(0.5*S0);
```

```
    if C_s(n)<0
       C_s(n) = 0;
    end
end



X_act(1) = N;
X_act(2:length(t)-2) = X_act(2:length(t)-2)*0.004;
figure(1);
plot(t(1:n-2), X_act(1:n-2));
title('Cell Biomass')
xlabel('t [hrs]');
ylabel('Active Cells [g/L]');

figure(2);
plot(t(1:n-2), C_s(1:n-2));
title(sprintf('Sugar Concentration'));
xlabel('t [hrs]');
ylabel('C_s [g/L]');

figure(3);
plot(t(1:n-2), C_e(1:n-2));
title('Ethanol Concentration')
xlabel('t [hrs]');
ylabel('C_e [g/L]');


%============================== Non-recursive probability calculations

    %************************************************************************%
    %Calculating W, the initial cell mass distribution of the inoculated cells%
    %************************************************************************%
    m = 0:0.01E-12:1E-11;
    epsilon = 3*sqrt(2)*10^-13;
    t = 0;
    W = 0.1.*(m./epsilon).^5.*exp(-m./epsilon).*10.^24;

    figure(4);
    plot(m.*1E12, W);
    title(sprintf('Cell Mass Distribution at a t = %d sec', t))
    xlabel('mass [picograms]')
    ylabel('number of yeast cells')


%************************************************************************%
%Calculating the Transition probability (Gamma) and the individual growth rate%

%************************************************************************%

    R = 5E-6;       %meters
    rho = 1.01;     %g/cm^3
    K_sL = 0.02;    %g/L
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```
    mu_cL = 1;       %hr^-1
    m_cL = 3E-12;    %g
    phi = 6E-5;      %g/cm^2?hr
    C_s = 0.03;      %g/L

    rprime = 2/(R*rho)*phi*C_s.*m./(K_sL+C_s);  %Calculates r' (Equation 12)
    rdubprime = mu_cL.*m;                       %Calculates r" (Equation 13)
    r = rprime - rdubprime;                %Calculates r from r' and r" (Eq. 11)


    L = 2/(epsilon*sqrt(pi)).*exp(-((m-m_cL)./epsilon).^2)./erfc((m-
    m_cL)./epsilon).*r; %Transition probability (Equation 10)

    figure(5);
    plot(m, L);
    title('Transition Probability')
    xlabel('cell mass')
    ylabel('Gamma(m)')

    %*************************************************************%
    %Calculate the Mass distribution denstiy of the daughter cells%
    %*************************************************************%

    m_new = input('Enter arbitrary parent mass to determine daughter cell mass
probabilities: ');
    p = 30.*m.^2.*(m_new - m).^2./(m_new).^5;

    figure(6)
    plot(m, p);
    title(sprintf('Probability of Daughter Cell Having mass, m, with Parent
Having mass, %d', m_new))
    xlabel('mass of daughter cell (grams)')
    ylabel('p(m, m_new)')

%============================== Determine when to stop the fermentation

t = 0:3:200;
t0 = length(t)-10;          %last time points before the end of the fermentation
tf = length(t)-2;

final_t = t(t0:tf);         %vectorized for input into polyfit
final_C_e = C_e(t0:tf);     %vectorized for input into polyfit

extrapolation = polyfit(final_t, final_C_e, 1); %last ten values

stop_ferment = 0.95*extrapolation(2);      %Calculates 95% of the max EtOH conc.

for n = 1:length(C_e)     %Determines time index at which 95% EtOH conc. reached
    TOL = 1;

    if abs(C_e(n)-stop_ferment) < TOL
        break
    end
```

```
end

t = 0:3:200;

fprintf('To achieve a 95 percent ethanol yield and maximize efficiency, \nstop
the fermentation at %d\n hours', t(n))
```