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Modelling batch and continuous fermentation by a crabtree negative yeast

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Background

Two different models were found for the production of ethanol by a negative crabtree yeast, which were based on Monod kinetics. The models were used for simulation of both batch process as well as continuous process. Provided data of experiments were used to analyse the monod models, by making the models fit the data points. Aeration limits were considered, since the cells most likely do a mix of aerobic growth and fermentative production of ethanol.

1. Aerobic batch growth

A simple Monod kinetic formula, Equation 1, was used to fit the data points during aerobic growth. This was performed by using the given stoichiometric formula given for the process to create a simple black-box model. The simulation can be seen in Figure 1. Parameters which were estimated through the Monod kinetic simulation can be seen in Table 1.

$$\mu = \frac{\mu_{max} * s}{Ks + s} \qquad \text{Equation 1}$$

Table 1. Values for aerobic constants found with the Monod kinetic model of Equation 1.

Ks [g/L]	Y_{sx} [g/g]	μ_{max} [/h]
0.1	0.4	0.5

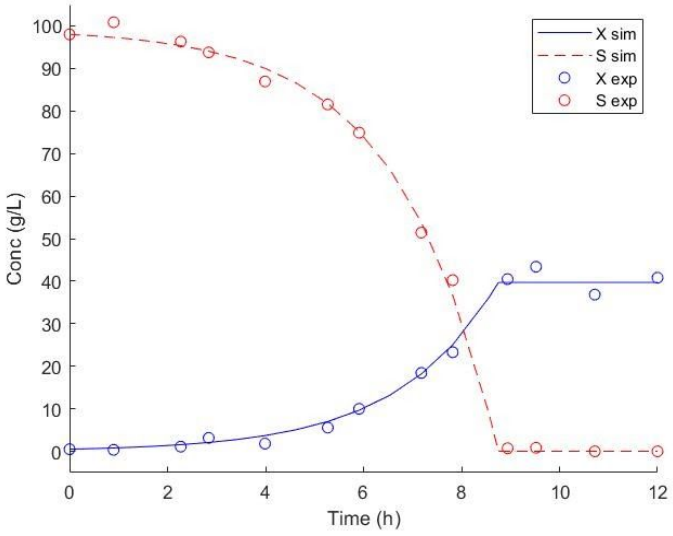

Figure 1. Shows the simulation of fitting data to the aerobic Monod kinetic model. The circles are the data found experimentally.

The μ_{max} found holds a reasonable value as well as it is found through data points fitting the chosen Monod kinetic model well, as can be seen in Figure 1. The yield of biomass per substrate is a reasonable value, if one compares it to achieved values of experiments and industry (Villadsen et. al, 2011). The model fits the data well, which indicates that the values found are reasonable. The form of the graph in Figure 1, is logical due to the fact that cells are growing exponentially and at a specific

point the cell growth stops. This occurs at the same time as the substrate is consumed. After a while the cells should start to die, but the Monod model does not capture death phase because it lacks the cell maintenance term.

2. Anaerobic batch growth

The same simple Monod kinetic model which was used in the first question was used in this simulation as well to fit the data available. As a difference from the previous one, this simulation referred to an anaerobic fermentation. By that oxygen was limited and therefore ethanol was produced by the yeast. The product formation can be seen in Figure 2, which shows the data fitted to the model, both for substrate consumption, cell growth and product formation. The best fit was found by using the parameters shown in Table 2.

Table 2. Values for anaerobic constants found with the Monod kinetic model.

Ks [g/L]	Y_{sx} [g/g]	μ_{max} [/h]	Y_{se} [g/g]
0.1	0.2	0.4	0.5

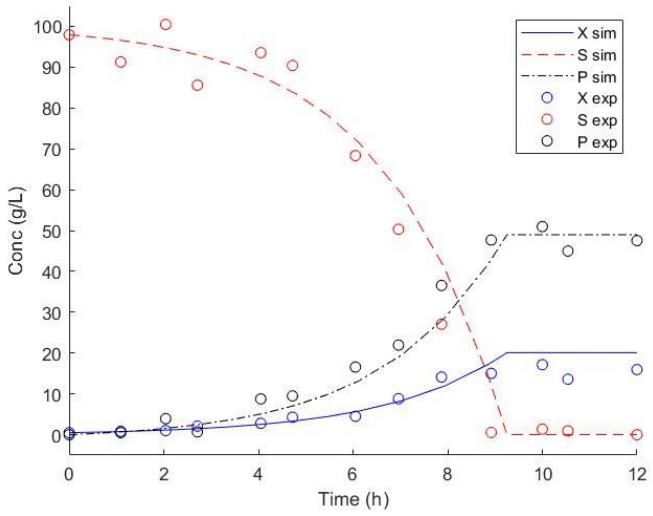

Figure 2. Shows the simulation of fitting data to the anaerobic Monod kinetic model. The circles are the data found experimentally.

The biomass formation do grow exponentially until substrate is depleted which is reasonable for the same explanation as the one of during the aerobic process. The product concentration follows the pattern of biomass formation. Ethanol production has a longer lag phase than biomass because ethanol requires a sufficient biomass concentration to be produced.

3. Oxygen limited growth in batch

To create a model with oxygen as a limiting substrate, we used a modified monod model, summing respiratory and fermentative growth terms. The respiratory growth term includes oxygen as a limiting substrate alongside substrate, and fermentative growth term includes an oxygen inhibition term that gets larger with less oxygen. The oxygen concentration in the broth is the combination of aerobic consumption and mass transfer from sparging. The aerobic consumption of oxygen is given by the

yield coefficient of oxygen per biomass multiplied with the aerobic biomass growth. The mass transfer of oxygen into the broth is given by the oxygen transfer rate, OTR, shown in equation 2.

$$OTR = K_L a * (C_o^* - C_o) \qquad \text{Equation 2}$$

In Figure 3, cell growth is exponential and primarily aerobic when there is both substrate and oxygen, which are then exponentially depleted because there is no feed. As soon as oxygen is depleted around 6 hours, fermentation becomes dominant and ethanol (P) is produced. Biomass growth continues via fermentation. When substrate is depleted, fermentation stops as well whatever respiration occurs on the sparged air, so cell and product concentrations become static. Because cell growth halts and all respiration ceases, the reactor is saturated with oxygen from sparged air.

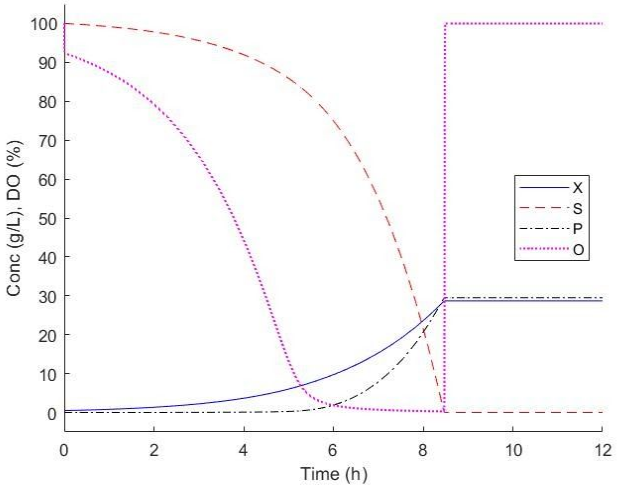

Figure 3. Shows the simulation of oxygen limited growth in batch reactor.

Oxygen depletion can be avoided by reducing the initial substrate concentration. In Figure 4, the initial substrate concentration is reduced to 25 g/L from 100 g/L. The cells consume this substrate more quickly, and so the substrate is depleted before oxygen, unlike in Figure 3 where the opposite occurs. Because there is always oxygen present with substrate, the cells prefer respiration, and so only a small quantity of ethanol is formed through fermentation when oxygen concentration is at its minimum. Thus, reducing substrate concentration so that it becomes the main limiting substrate can prevent unwanted fermentative byproducts.

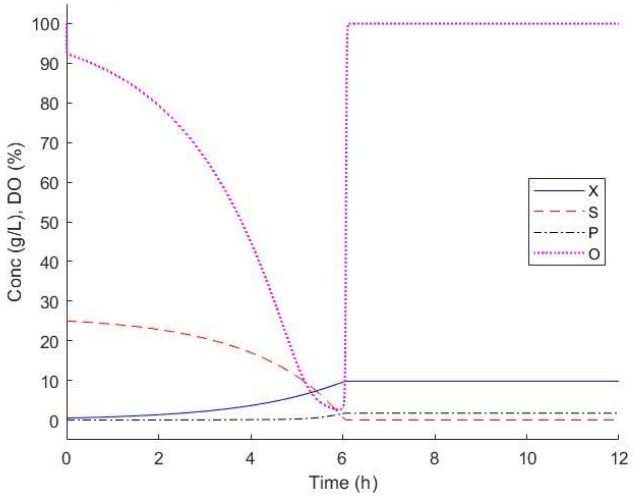

Figure 4. Mixed aerobic and fermentative growth with reduced initial substrate concentration.

4. Oxygen limited growth in chemostat

The mixed aerobic and fermentative growth model was modified to include inlet and outlet streams. The model was used to find the steady state concentrations of each component at a variety of dilution rates. At low dilution rates, cells have time to consume all the substrate aerobically with excess oxygen being sparged into the tank. As the dilution rate increases, oxygen transfer is not fast enough to support fully aerobic growth, so some ethanol is produced from fermentation. Because aerobic growth is more productive, biomass concentration decreases (Villadsen et. al, 2011). As oxygen concentration approaches 0, with the cells consuming oxygen faster than it is sparged, the ethanol concentration peaks with the cells primarily fermenting. However, around a dilution rate of 0.4 h^{-1}, the concentration of biomass and ethanol drops while the concentration of substrate rises sharply. This represents the beginning of washout. It makes sense to see washout starting around this dilution rate because, in a chemostat, dilution rate is equal to the biomass growth rate, μ. μ_{max} for fermentation was set to 0.4 h^{-1}, which means that the washout dilution rate for fermentative growth is 0.4 h^{-1}. The behavior is somewhat complicated having two different μ_{max} for respiration and fermentation, but the ethanol production drops off at the washout dilution rate for fermentative growth, which seems reasonable, and substrate concentration rises as cell concentration plunges. However, because the dilution rate for aerobic growth is 0.5 h^{-1}, not all the cells are washed out at 0.4 h^{-1}. This is purely a result of combining the aerobic and anaerobic models while keeping separate parameters optimized for each. At 0.5 h^{-1}, the biomass concentration drops to 0, indicating full washout, and accordingly oxygen concentration rises to the maximum dissolved oxygen concentration.

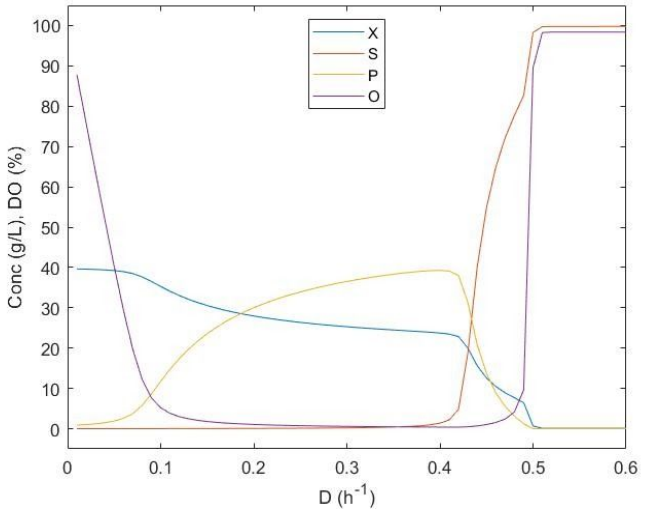

Figure 5. Shows the simulation of oxygen limited growth in chemostat.

References

Villadsen. J, Nielsen. J, Lidén. G, *Bioreaction Engineering Principles*, Springer US, 2011.