Effect of temperature on enzyme activity. Activation energy acts on both the conversion step and the deactivation step simultaneously.
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Let us consider Michaelis-Menten kinetics of enzyme reaction.

$$S + E \underset{}{\overset{K_m}{\longleftrightarrow}} ES \xrightarrow{k_2} P + E$$ ... Conversion of substrate to product

$$E \xrightarrow{k_d} E_{inactive}$$ ... Enzyme deactivation

Gas law constant: $R := 0.0019872 \cdot \frac{kcal}{mole \cdot K}$

Activation energy of the conversion step. Pre-exponential factor (Arrhenius constant)

Conversion step $E_a := 11 \cdot \frac{kcal}{mole}$ $A := 10^8 \cdot min^{-1}$

Deactivation step $E_d := 70 \cdot \frac{kcal}{mole}$ $B := 10^{49} \cdot min^{-1}$

Activation temperature

Conversion step $T_a := \frac{E_a}{R}$ $T_a = 5.535 \cdot 10^3 \cdot K$

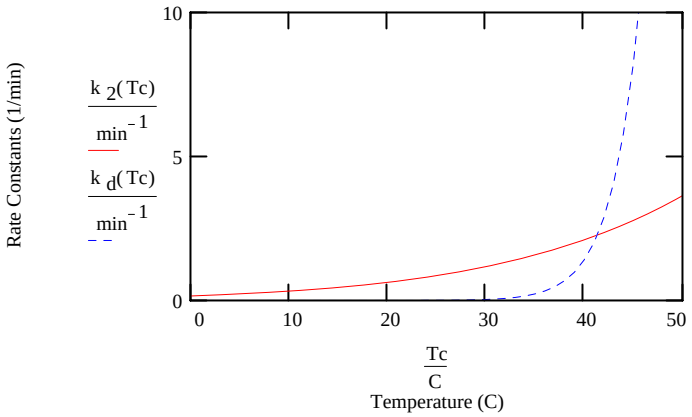
Deactivation step $T_d := \frac{E_d}{R}$ $T_d = 3.523 \cdot 10^4 \cdot K$

Reaction rate "constants"

Conversion step $k_2(Tc) := A \cdot \exp\left(-\frac{T_a}{Tk(Tc)}\right)$ $k_2(37 \cdot C) = 1.774 \cdot min^{-1}$

Deactivation step $k_d(Tc) := B \cdot \exp\left(-\frac{T_d}{Tk(Tc)}\right)$ $k_d(37 \cdot C) = 0.473 \cdot min^{-1}$

$Tc := 0 \cdot C .. 50 \cdot C$



Temperature sensitivity at a higher temperature. The deactivation rate constant k_d increases much faster compared to the conversion rate constant k_2.

Conversion step $k_2(40\cdot C) = 1.792 \cdot k_2(30\cdot C)$ The value of k_2 at 40°C is 1.8 times that at 30°C.

Deactivation step $k_d(40\cdot C) = 40.879 \cdot k_d(30\cdot C)$ The value of k_d at 40°C is 41 times that at 30°C.

The number of fold of increase depends on the temperature difference (T_1 and T_2) and the exponential term (i.e., activation energy). It does not depend on the Arrhenius constants.

$$fold(T_a, Tc_1, Tc_2) := \exp\left[-T_a\cdot\left(\frac{1}{Tk(Tc_2)} - \frac{1}{Tk(Tc_1)}\right)\right]$$

Conversion step $fold(T_a, 30\cdot C, 40\cdot C) = 1.792$

Deactivation step $fold(T_d, 30\cdot C, 40\cdot C) = 40.879$

The enzyme suffers even more severely as we raise temperature from 30°C to 50°C.

Conversion step $fold(T_a, 30\cdot C, 50\cdot C) = 3.096$

Deactivation step $fold(T_d, 30\cdot C, 50\cdot C) = 1.328\cdot 10^3$

Derivation of the reaction rate expression.

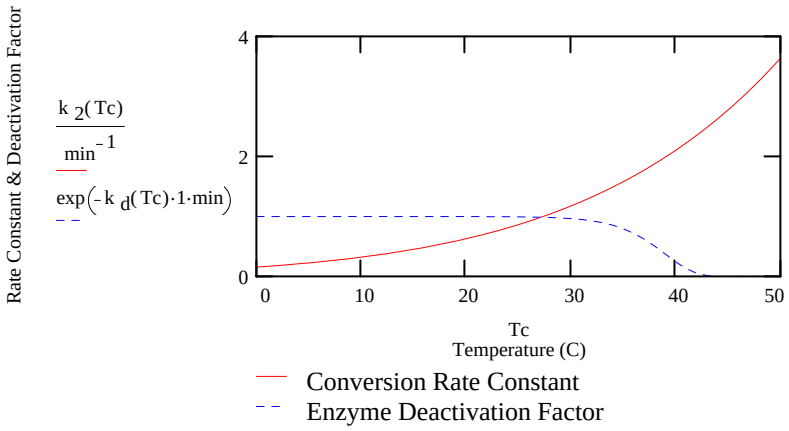
1. With K_m<<S, the rate of conversion is does not depend on K_m or s. $\frac{dp}{dt} = v = v_m$
2. v_m is a combination of the conversion rate constant and enzyme level. $v_m = k_2\cdot E$
3. Enzyme deactivates with time in a first-order fashion. $E = E_0\cdot\exp(-k_d\cdot t)$

Combining 1, 2, & 3, we derive reaction rate as a function of the temperature in °C and time t.

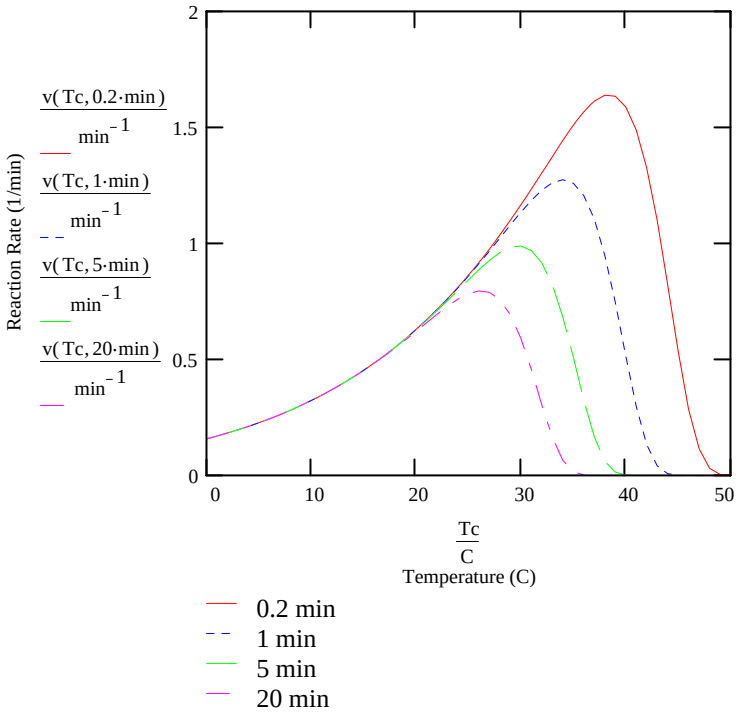
$E_0 := 1$ $\quad v(Tc, t) := k_2(Tc)\cdot E_0\cdot\exp(-k_d(Tc)\cdot t)$

The reaction rate v is affected by two opposing factors:

1) k_2 , which raises it ↑, as temperature increases ↑ and
2) $\exp(-k_d\cdot t)$, which lowers it ↓, as temperature increases ↑.



At a low temperature, the enzyme activity increases with temperature because the activation energy acts mainly on the conversion rate constant k_2. On the other hand, at a high temperature, the enzyme quickly loses its activity because the activation energy raises the deactivation rate constant k_d. The next plot shows the combined effect of these two factors.



Product concentration. The following expression is valid in general.

$$p(Tc,t) := \int_{0\cdot min}^{t} v(Tc,\tau)\, d\tau$$

Analytical solution exists for the isothermal case, when K_m<<s, and when s is in excess.

$$\int_0^p 1\, dP = \int_{0\cdot min}^{t} k_2(Tc)\cdot E_0\cdot \exp\left(-k_d(Tc)\cdot\tau\right) d\tau$$

Integrating by choosing |Symboli|Evaluate|Evaluate Symbolically| yields:

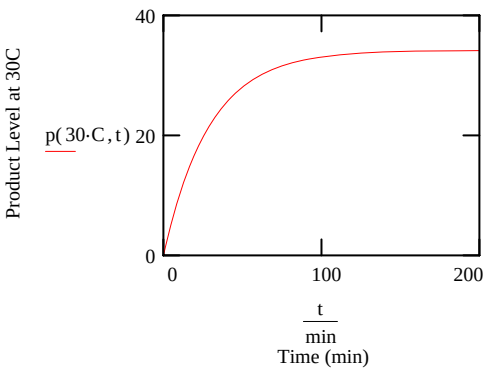
$$p = \frac{-1}{k_d(Tc)}\cdot\exp\left(-k_d(Tc)\cdot t\right)\cdot k_2(Tc)\cdot E_0 + \frac{1}{k_d(Tc)}\cdot k_2(Tc)\cdot E_0$$

which simplifies to:

$$p = \frac{k_2(Tc)}{k_d(Tc)}\cdot E_0\cdot\left(1-\exp\left(-k_d(Tc)\cdot t\right)\right)$$

$$p(Tc,t) := \frac{k_2(Tc)}{k_d(Tc)}\cdot E_0\cdot\left(1-\exp\left(-k_d(Tc)\cdot t\right)\right)$$

$$t := 0\cdot min, 1\cdot min .. 200\cdot min$$



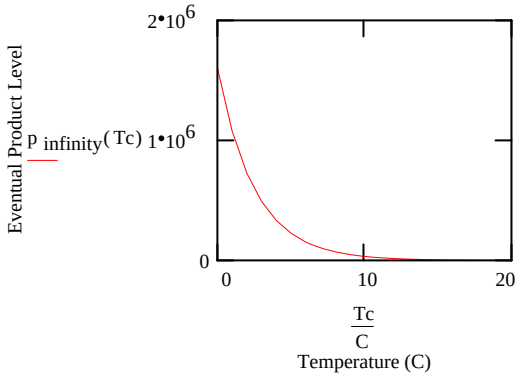
In a batch reactor, the enzyme eventually becomes completely deactivated, and there is no further conversion. The dynamic time constant for the product formation process is $1/k_d$. The eventual product level depends on the ratio of k_2 and k_d, and is proportional to the initial amount of enzyme.

as $t \to \infty$, $p_{infinity}(Tc) := \frac{k_2(Tc)}{k_d(Tc)} \cdot E_0$

Example: $p_{infinity}(30 \cdot C) = 34.205$

Maximum amount of product formed per unit enzyme. $\frac{p_{infinity}}{E_0} = \frac{k_2}{k_d}$

$Tc := 0 \cdot C .. 20 \cdot C$



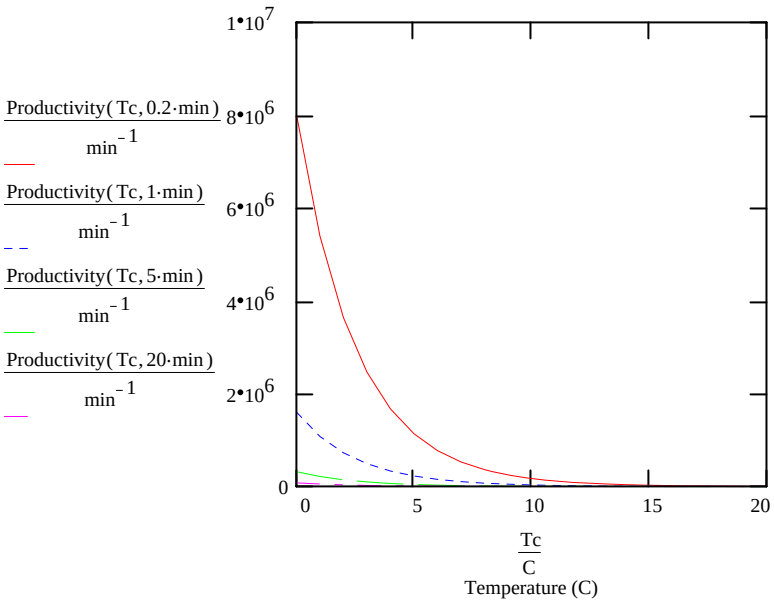
Objective Function #1. The above plot shows that the enzyme stays active longer and converts more substrate into product per unit of enzyme at lower temperature. The maximum amount of product formed per unit enzyme, i.e., $p_{infinity}/E_0$, also has the same monotonic trend. However, conversion from p=0 to $p=p_{infinity}$ is accomplished at a slower rate at a lower temperature, the time constant of the process dynamics being $1/k_d$, as shown in the p vs. t plot. Thus, we define productivity generally as the amount of product formed per unit enzyme lost per unit time.

$$\text{Productivity} = \frac{p_{infinity}}{E_0 \cdot \frac{1}{k_d}} = k_2$$

As shown in a previous plot of k_2 versus temperature, k_2 increases monotonically with temperature. Thus, productivity is higher at a higher temperature. If we assume that the residual enzyme is fully recoverable at no cost -- which is not really a realistic assumption, then the following could be a definition of productivity.

$$\text{Productivity}(Tc,t) = \frac{\text{amount\_product\_formed}}{\text{amount\_of\_enzyme\_consumed} \cdot \text{time}} = \frac{p(Tc,t)}{E_0 \cdot \left(1 - \exp\left(-k_d(Tc) \cdot t\right)\right) \cdot t} = \frac{k_2(Tc)}{k_d(Tc) \cdot t}$$

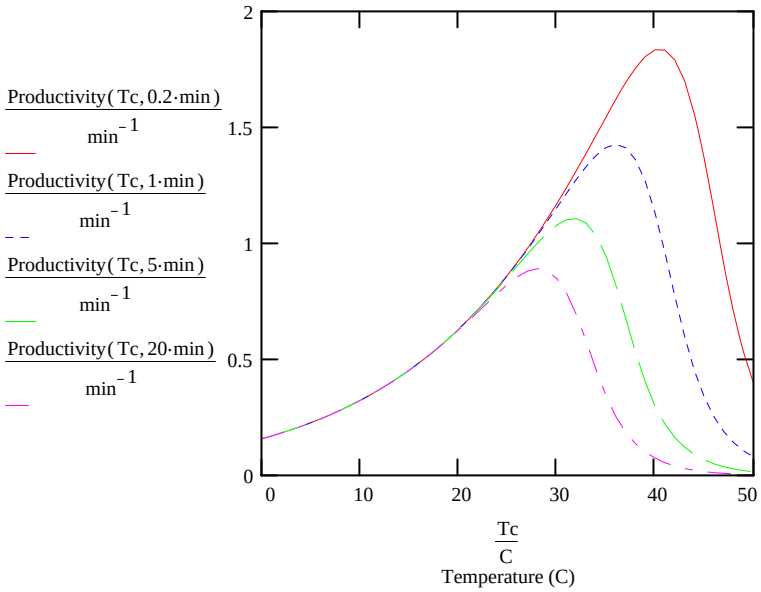
$$\text{Productivity}(Tc,t) := \frac{k_2(Tc)}{k_d(Tc) \cdot t}$$



Objective Function #2. Assuming that the residual enzyme is unrecoverable -- which is more realistic, the following is a reasonable definition of productivity.

$$\text{Productivity}(Tc,t)=\frac{\text{amount\_product\_formed}}{\text{initial\_amount\_of\_enzyme\_}\cdot\text{time}}=\frac{p(Tc,t)}{E_0\cdot t}=\frac{k_2(Tc)}{k_d(Tc)\cdot t}\cdot\left(1-\exp\left(-k_d(Tc)\cdot t\right)\right)$$

$$\text{Productivity}(Tc,t):=\frac{p(Tc,t)}{E_0\cdot t}\qquad Tc:=0\cdot C..50\cdot C$$



The above plot is similar to the v versus temperature plot. We see that reacting quickly at a high temperature is the most productive. In practice, we need to carry out the enzyme reaction in an aqueous phase, so the higher temperature limit will be limited by the boiling point of the solution. There may be thermal degradation of both the substrate and the product. Furthermore, as the rate of reaction increases, the reaction will become mass transfer limited, rather than reaction kinetics limited, even in the solution phase. This formulation is flawed because it does not take into consideration the cost of the enzyme -- if we are making a product at a cost, we lose more money by producing faster!

Objective Function #3. A better measure of productivity is to maximize the profit from operating a bioreactor of a fixed size per unit time. This means considering the market value of the product, minus the enzyme and substrate cost and the operating cost. Thus, at a low temperature, we make enough product to offset the cost of the enzyme but the production rate is too slow; whereas, at a high temperature, the production rate is high, but the amount of product produced per unit enzyme is too low to offset the cost of the enzyme. Instead of maximizing the productivity of the product, we maximize the productivity of $$$, i.e., profit.

Value of the substrate relative to the product: $\alpha_s := 0.1$

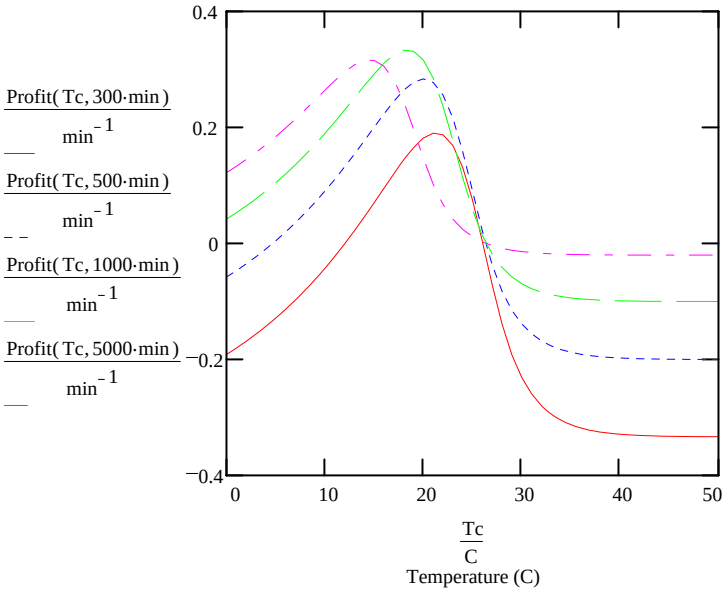
Value of the enzyme relative to the product: $\alpha_E := 100$

$$\text{Profit} = \frac{1}{\text{time}} \cdot \left(\text{product\_formed} - \alpha_s \cdot \text{substrate\_consumed} - \alpha_E \cdot \text{enzyme}\right)$$

$$\text{.} = \frac{1}{\text{time}} \cdot \left[\left(1 - \alpha_s\right) \cdot \text{product\_formed} - \alpha_E \cdot \text{enzyme}\right]$$

$$\text{Profit}(Tc, t) := \frac{E_0}{t} \cdot \left[\left(1 - \alpha_s\right) \cdot \frac{k_2(Tc)}{k_d(Tc)} \cdot \left(1 - \exp\left(-k_d(Tc) \cdot t\right)\right) - \alpha_E\right]$$

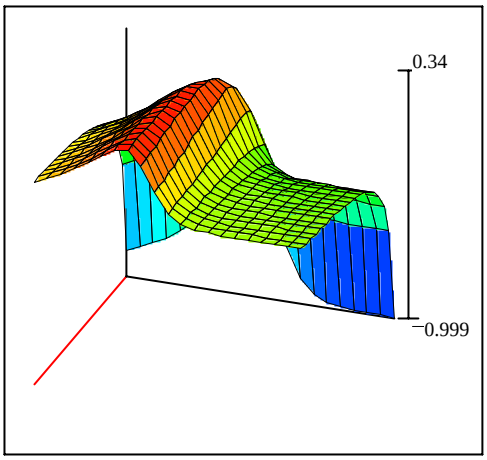
$$\text{Profit}(Tc, t) := \frac{1}{t} \cdot \left[\left(1 - \alpha_s\right) \cdot p(Tc, t) - \alpha_E \cdot E_0\right]$$



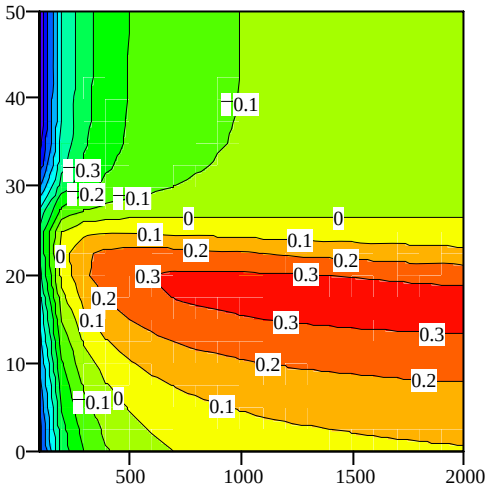
3-D surface plot $j := 0 .. 20$ $Tc_j := 2.5 \cdot C \cdot j$

$i := 0 .. 19$ $t_i := 100 \cdot (i + 1) \cdot min$

$$profit_{i,j} := \frac{Profit(Tc_j, t_i)}{min^{-1}}$$



profit



profit

⇓ Increasing time from 100 to 2000 min.
⇒ Increasing temperature from 0 to 50°C.

⇑ Increasing temperature from 0 to 50°C.
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The above plot clearly shows that there is a maximum profit. At the maximum, all partial derivatives are equal to zero. Unfortunately, the Given-Find function does not work well when the units do not match -- a shortcoming of Mathcad. (Mathcad does not allow us to place apples and oranges in the same vector. Because Find(Tc,t) results in a vector of temperature and time, Mathcad complains about incompatible units.) We redefine the variables without the physical units.

$$A := \frac{A}{min^{-1}} \qquad T_a := \frac{T_a}{K} \qquad k_2(Tc) := A \cdot \exp\left(-\frac{T_a}{Tc + 273.15}\right)$$

$$B := \frac{B}{min^{-1}} \qquad T_d := \frac{T_d}{K} \qquad k_d(Tc) := B \cdot \exp\left(-\frac{T_d}{Tc + 273.15}\right)$$

$$p(Tc, t) := \frac{k_2(Tc)}{k_d(Tc)} \cdot E_0 \cdot \left(1 - \exp\left(-k_d(Tc) \cdot t\right)\right)$$

$$Profit(Tc, t) := \frac{1}{t} \cdot \left[\left(1 - \alpha_s\right) \cdot p(Tc, t) - \alpha_E \cdot E_0\right]$$

$Tc := 20$ $t := 1000$ ... initial guesses

Given $\frac{d}{dTc} Profit(Tc, t) = 0$ $\frac{d}{dt} Profit(Tc, t) = 0$ $\begin{pmatrix} Tc_{opt} \\ t_{opt} \end{pmatrix} := Find(Tc, t)$

$Tc_{opt} = 17.275$ $t_{opt} = 1.575 \cdot 10^3$ $Profit\left(Tc_{opt}, t_{opt}\right) = 0.341$

☹ Mathcad v7 fails to find a solution with the "Given-Find" block by solving d/d?=0, but "Given-Minerr" block is o.k., although the solutions are not exactly identical.

Given $\text{Profit}(Tc, t) = 0.5 \qquad 0 = 0$

$$\begin{pmatrix} Tc_{opt} \\ t_{opt} \end{pmatrix} := \text{Minerr}(Tc, t)$$

$Tc_{opt} = 17.35 \qquad t_{opt} = 1.544 \cdot 10^{3} \qquad \text{Profit}(Tc_{opt}, t_{opt}) = 0.341$

Of course, the optimum temperature and the optimum reaction time depend on the model parameters and the cost of substrate and enzyme relative to the value of the product. In optimization, correct formulation of the objective function is extremely important, as the solution depends on the formulation. If formulated incorrectly, we have garbage in and garbage out.

Some temperature definitions

$C \equiv K \qquad Tk(c) \equiv c + 273.15 \cdot K$ Check: $Tk(100 \cdot C) = 373.15 \cdot K$