

Analytical Determination of the Optimal Temperature Profiles for the Reactions with Parallel Deactivation of Enzyme Encapsulated Inside Microorganisms Cells

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A batch biotransformation process running in the presence of microorganisms cells revealing a specified enzyme activity has been considered. The parallel deactivation of enzyme has been taken into account. An analytical solution determining the process course under isothermal conditions and at an optimal temperature profile has been presented.

The advantages resulting from the application of the optimal temperature profile with respect to the isothermal conditions in terms of the duration time of the process have been assessed. The presence of the cell membrane has also been taken into account.

Key words:

Biocatalyst deactivation, biochemical reactors, optimization, reaction engineering, variational calculus

Introduction

Biotransformation processes that run in the presence of native enzymes or microorganisms are traditionally carried out in batch reactors. Temperature is the most important decisive variable in such types of reactors that affect both the reaction rate as well as enzyme deactivation. Therefore, the most pertinent problem in control of their performance is the choice of an appropriate temperature policy. The simplest and the most frequently applied way to carry out such processes is maintaining isothermal conditions while the problem to adopt an optimal temperature profile is important mainly from the cost-effective production point of view.^{1–5}

The biotransformations can be carried out in the presence of a native enzyme or an enzyme encapsulated in a whole microbial cell. In spite of diminished selectivity, the latter is particularly advantageous since the natural environment increases enzyme stability, and thus decreases enzyme deactivation.

The analysis of the optimal temperatures given in literature has shown that a kinetics of (bio-) catalyst deactivation and mutual relations between the activation energies exert the decisive effect.^{6,7} The majority of the processes analyzed from the optimal point of view take into consideration the simplest deactivation model, i.e. a first order deactivation independent of substrate concentration.^{8–10}

Less often, the optimal conditions have been considered for processes with a catalyst deactivation

dependent upon substrate concentration.^{11–13} The process of hydrogen peroxide decomposition in the presence of the native catalase or contained in the *Saccharomyces cerevisiae* yeast cells belongs to the latter. In the case of catalase, readily available *Saccharomyces cerevisiae* cells are of special interest because of their broad application in the food industry.^{14–16}

The determination of the optimal temperature for a biotransformation with an enzyme encapsulated in microbial cells is significantly more difficult than that for a native enzyme. This is because in the former it is necessary to consider a substrate and product transport through a cell membrane.

The effect of internal and external diffusional resistances on the overall rate of the processes carried out in the presence of immobilized enzymes have been reported in literature.^{17–21} In most cases, the processes have been analyzed for isothermal conditions,²² either without considering deactivation of the applied biocatalyst^{23–26} or assuming first order deactivation.^{27–29} The models have been solved by using either the combined analytical and numerical techniques^{30–33} or the latter alone.³⁴ So far only Grubecki i Wójcik³⁵ have presented an analytical solution to the optimal temperature profile for an enzymatic reaction carried out in the presence of microorganism cells. They have analyzed the process with enzyme deactivation independent of the substrate concentration.

Moreover, no research has been reported on the optimal temperature control for a biotransformation

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process in which diffusional phenomena of a deactivating biocatalyst had been considered.

The aim of the present work is to indicate the possibility to obtain an analytical solution to the optimal temperature profile for the batch bio-transformation process with parallel deactivation of enzyme encapsulated inside microorganisms cells and accounting for the diffusive phenomena across the cell membrane.

Also, an effect of the presence of the latter on the mentioned temperature profiles and the optimal time of the process have been considered.

Analysis has been conducted for a particular case, in which the activation energy for the permeation process through cell membrane E_p is equal to that of the reaction E_R ($E_p = E_R$). However, based on this analysis, it is possible to indicate the general rules taking place during the accomplishment of the processes in which the enzyme is separated from the reaction environment by a cell membrane.

In addition, these considerations have been extended into an analysis of the isothermal conditions as an indispensable element needed for optimization analysis of any process conducted in a batch reactor or plug flow reactor.

Mathematical model

The course of any enzymatic process is dependent on a number of effects that can be examined independently. These effects are difficult to measure, especially in the case of whole cells. When formulating the mathematical model of the analyzed process the following assumptions made:

– Substrate transport across the cell membrane is an equimolar diffusion process affecting the overall process rate

$$-\frac{dC_s}{dt} = k_p(C_s - C_{SIn}) \quad (1)$$

where C_s , C_{SIn} , k_p determine the substrate concentration, substrate concentration inside the cells, and overall mass transfer coefficient across the cell membrane, respectively.

– The rate of reaction r_s occurring inside the microorganism cells is described by the Michaelis-Menten kinetics,

$$r_s = k_R \frac{C_E C_{SIn}}{K_M + C_{SIn}} \quad (2)$$

where K_M denotes Michaelis-Menten constant for reaction.

– An effect of substrate concentration on the enzyme deactivation rate, in agreement with Do and Weiland theory,³⁶ taken into account

$$-\frac{dC_E}{dt} = k_D \frac{C_E C_{SIn}}{(K_D + C_{SIn})} \quad (3)$$

where K_D denotes Michaelis-Menten constant for deactivation.

– The effect of temperature on the reaction rate constant k_R , deactivation rate constant k_D and the overall mass transfer coefficient across the cell membrane k_p is expressed by the Arrhenius equation

$$k_i = k_{i0} \exp\left(-\frac{E_i}{RT}\right) \quad (i = D, P, R) \quad (4)$$

where E_i and k_{i0} denote the activation energy and frequency factor for deactivation ($i = D$), permeability of the cells membrane ($i = P$) and for reaction ($i = R$).

– The Michaelis-Menten constant for reaction K_M and deactivation K_D is assumed to be independent of temperature because its variation with this parameter can be neglected.^{16,25,27,35,37–43}

– The cells are built with a membrane of small permeation or contain enzyme of high activity so, as a consequence, the following inequality holds $C_{SIn} \ll K_D$, $C_{SIn} \ll K_M$,

– In spite of the decrease in enzyme activity the rate of the substrate transport across the cell membrane is equal to the reaction rate inside the cells (quasi-steady state assumption).

In the situation after introducing dimensionless variables $\bar{C}_j = C_j/C_{j0}$ ($j = E, S$) and $\bar{K}_l = K_l/C_{S0}$ ($l = D, M$), the mathematical model, together with the initial conditions, will take the following form

$$-\frac{d\bar{C}_S}{dt} = \frac{k'_R}{\bar{K}_M} \frac{\bar{C}_E \bar{C}_S}{\left(1 + \frac{k'_R}{k_p \bar{K}_M} \bar{C}_E\right)} \quad (5a)$$

$$\bar{C}_S(t=0) = \bar{C}_{S0} \quad \bar{C}_S(t=t_f) = \bar{C}_{Sf}$$

$$-\frac{d\bar{C}_E}{dt} = \frac{k_D}{\bar{K}_D} \frac{\bar{C}_E \bar{C}_S}{\left(1 + \frac{k'_R}{k_p \bar{K}_M} \bar{C}_E\right)} \quad (5b)$$

$$\bar{C}_E(t=0) = \bar{C}_{E0} \quad \bar{C}_E(t=t_f) = \bar{C}_{Ef}$$

where $k'_R = k_R C_{E0}/C_{S0}$.

It should be pointed out that the mathematical model formulated above is also related to enzymes immobilized by encapsulation and the analyzed deactivation mechanism concerns with many enzymes currently applied in industrial practice.^{44–46}

Solution of the model

When solving the above formulated model a process carried out under isothermal conditions and at the optimal temperature profile, guaranteeing maximum conversion $\alpha_f = 1 - \bar{C}_{Sf}$ or minimum time $t_{f,opt}$ indispensable for its achieving, has been considered. From the mathematical point of view, both these variants of the optimal solutions are equivalent.⁴⁷

In industrial practice a typical situation is encountered when a desired process is conducted starting from the initial substrate concentration $\bar{C}_S(t = t_0) = \bar{C}_{S0}$ and the initial biocatalyst activity $\bar{C}_E(t = t_0) = \bar{C}_{E0}$ (in particular $\bar{C}_{E0} = 1, \bar{C}_{S0} = 1$) and terminating at the fixed final values of these state variables $\bar{C}_{Sf}, \bar{C}_{Ef}$.

Isothermal conditions

In analyzing the course of the process under the isothermal conditions, our target is to find the temperature of the reaction T_{isot} and the time of its completion $t_{f,isot}$. To that end, it is necessary to eliminate time from the equations of state (5a) and (5b). To do this, the equations were divided side by side and then integrated from the given initial conditions $\bar{C}_{S0} = 1, \bar{C}_{E0} = 1$ to obtain

$$\bar{C}_S = 1 - \vartheta(T)(1 - \bar{C}_E) \quad (6)$$

$$\text{where } \vartheta(T) = \frac{k'_R(T)\bar{K}_D}{k_D(T)\bar{K}_M}.$$

Assuming in this equation $\bar{C}_S = \bar{C}_{Sf}, \bar{C}_E = \bar{C}_{Ef}$, after the appropriate transformation, the following expression describing the searched temperature is obtained

$$T_{isot} = \left\{ \frac{R}{(E_D - E_R)} \ln \left[k_{DS} \frac{\bar{K}_D}{\bar{K}_M} \frac{(1 - \bar{C}_{Sf})}{(1 - \bar{C}_{Ef})} \right] \right\}^{-1} \quad (7)$$

where $k_{DS} = k_{D0}C_{S0}/(k_{R0}C_{E0})$.

The process duration time under the analyzed conditions results directly from the integration of eq. (5b), after initial elimination of substrate concentration in it using eq. (6). For the temperature given by eq. (7), this leads to the following relations:

$$t_{f,isot} = k_{P0}^{-1} \left[k_{DS} \frac{\bar{K}_M}{\bar{K}_D} \frac{(1 - \bar{C}_{Sf})}{(1 - \bar{C}_{Ef})} \right]^{1/(E-1)} \cdot \left\{ k_{PM}(1 - \bar{C}_{Sf}) \frac{\ln(\bar{C}_{Sf}/\bar{C}_{Ef})}{(\bar{C}_{Sf} - \bar{C}_{Ef})} - \right. \quad (8a)$$

$$\left. - \left[k_{DS} \frac{\bar{K}_M}{\bar{K}_D} \frac{(1 - \bar{C}_{Sf})}{(1 - \bar{C}_{Ef})} \right]^{\frac{(E_P/E_R-1)}{(E-1)}} \ln(\bar{C}_{Sf}) \right\}$$

for $\bar{C}_{Sf} \neq \bar{C}_{Ef}$ and

$$t_{f,isot} = k_{P0}^{-1} \left[k_{DS} \frac{\bar{K}_M}{\bar{K}_D} \right]^{1/(E-1)} \cdot \left\{ k_{PM} \frac{(1 - \bar{C}_{Ef})}{\bar{C}_{Ef}} - \left[k_{DS} \frac{\bar{K}_M}{\bar{K}_D} \right]^{\frac{(E_P/E_R-1)}{(E-1)}} \ln(\bar{C}_{Ef}) \right\} \quad (8b)$$

for $\bar{C}_{Sf} = \bar{C}_{Ef}$

where $k_{PM} = k_{P0}K_M/(k_{R0}C_{E0})$ and E denote activation energy quotient.

The second relation (eq. (8b)) is obtained by calculating the limit of the right side of eq. (8a) for $\bar{C}_{Sf} \rightarrow \bar{C}_{Ef}$.

Optimal temperature control

Optimal conditions provide the most advantageous solution for process control in view of the analyzed objective function. They have been determined based on the accomplishment of the optimization problem, which was aimed at finding a temperature profile $T_{stat}(t)$ such that for the initial conditions of the substrate concentration $\bar{C}_S(t = t_0) = \bar{C}_{S0}$ and the enzyme activity $\bar{C}_E(t = t_0) = \bar{C}_{E0}$ (in particular $\bar{C}_{E0} = 1, \bar{C}_{S0} = 1$) as well as the appropriate fixed final values of the state variables \bar{C}_{Sf} and \bar{C}_{Ef} will minimize the process duration time $t_{f,stat}$.

In solving the formulated problem a classical method of variational calculus⁴⁸ has been applied to yield directly a differential equation describing an optimal varying temperature. For the analyzed problem the following equation is obtained (see Appendix A):

$$\frac{dT}{dt} = T^2 \frac{R}{E_D E_R} [1 + X_T(b_1 + b_2 X_T)]^{-1} \cdot \left[E_D \frac{k_D}{\bar{K}_D} \bar{C}_S + (E_R + E_P X_T) \frac{k'_R}{\bar{K}_M} \bar{C}_E \right] \quad (9)$$

where

$$X_T = \frac{k'_R}{k_P \bar{K}_M} \bar{C}_E$$

$$b_1 = \frac{(E_D + E_P - E_R)(E_D E_R + E_D E_P - E_P E_R)}{E_D E_R (E_D - E_R)} - \frac{E_P}{(E_D - E_R)}$$

$$b_2 = \frac{E_P}{E_D E_R} (E_D + E_P - E_R)$$

The above expression, together with the state equations (5), composes the model of the process carried out under a stationary profile of optimal temperature. The effect of the formulated optimization problem can be classified as a two-point boundary value problem, which can only be solved using numerical methods.

For one case, the solution in question can be replaced by an analytical one. It does exist in the situation when the activation energy of the diffusion process through the cell membrane E_P is equal to that for the reaction E_R ($E_P = E_R$). In such case, the mathematical model (5), together with an equation for stationary profile of optimal temperature (eq. (9)) simplifies into the following relations:

$$\frac{d\bar{C}_S}{dt} = -\frac{k'_R}{\bar{K}_M} k_{PM} \frac{\bar{C}_E \bar{C}_S}{(k_{PM} + \bar{C}_E)} \quad (10a)$$

$$\bar{C}_S(t = t_0) = 1 \quad \bar{C}_S(t = t_f) = \bar{C}_{Sf}$$

$$\frac{d\bar{C}_E}{dt} = -\frac{k_D}{\bar{K}_D} k_{PM} \frac{\bar{C}_E \bar{C}_S}{(k_{PM} + \bar{C}_E)} \quad (10b)$$

$$\bar{C}_E(t = t_0) = 1 \quad \bar{C}_E(t = t_f) = \bar{C}_{Ef}$$

$$\frac{dT}{dt} = T^2 \left[\frac{R}{E_R} \frac{k_D}{\bar{K}_D} k_{PM}^2 \frac{\bar{C}_S}{(k_{PM} + \bar{C}_E)^2} + \frac{R}{E_D} \frac{k'_R}{\bar{K}_M} k_{PM} \frac{\bar{C}_E}{(k_{PM} + \bar{C}_E)} \right] \quad (10c)$$

Considering the right sides of equations (10), the last one can be given by

$$dT = -T^2 \left[\frac{R}{E_R} \frac{k_{PM}}{(k_{PM} + \bar{C}_E) \bar{C}_E} d\bar{C}_E + \frac{R}{E_D \bar{C}_S} d\bar{C}_S \right] \quad (11)$$

Integration of the above expression for the initial conditions T_0 , $\bar{C}_{E0} = 1$, $\bar{C}_{S0} = 1$ gives

$$T_{stat} [\bar{C}_{E,stat}(t), \bar{C}_{S,stat}(t)] = \left\{ \frac{1}{T_0} + \frac{R}{E_R} \ln \left[(k_{PM} + 1) \frac{\bar{C}_{E,stat} (\bar{C}_{S,stat})^{1/E}}{(k_{PM} + \bar{C}_{E,stat})} \right] \right\}^{-1} \quad (12)$$

After substituting eq. (12) to eq. (10a) and integrating within the limits of the initial value, an expression is obtained as follows

$$\bar{C}_{S,stat}(t) = \left[1 - \frac{(k_P)_{T_0}}{E(k_{PM} + 1)} t \right]^E \quad (13)$$

$(k_P)_{T_0}$ appearing in the above equation denotes the mass transfer coefficient through the cell membrane at initial temperature T_0 .

As a rule, in the optimizing procedure the initial process temperature T_0 is not known. Similarly as in the optimization problem, it can be determined after assuming the final substrate concentration $\bar{C}_{S,stat}(t = t_f) = \bar{C}_{Sf}$. Hence, based on eq. (13) we obtain

$$T_0 = \left\{ \frac{R}{E_R} \ln \left[\frac{k_{P0} t_f}{E(k_{PM} + 1)(1 - \bar{C}_{Sf}^{1/E})} \right] \right\}^{-1} \quad (14)$$

where t_f denotes the duration time of the process.

Substitution of the last equation into eqs. (12) and (13) yields

$$T_{stat} [\bar{C}_{E,stat}(t), \bar{C}_{S,stat}(t)] = \left\{ \frac{R}{E_R} \ln \left[\frac{k_{P0} t_f}{E(1 - \bar{C}_{Sf}^{1/E})} \frac{\bar{C}_{E,stat} (\bar{C}_{S,stat})^{1/E}}{(k_{PM} + \bar{C}_{E,stat})} \right] \right\}^{-1} \quad (15)$$

$$\bar{C}_{S,stat}(t) = \left[1 - (1 - \bar{C}_{Sf}^{1/E}) \frac{t}{t_f} \right]^E \quad (16)$$

As it follows from eq. (15), since the functions $\bar{C}_{S,stat}(t)$ and $\bar{C}_{E,stat}(t)$ are monotonically diminishing with time t , the stationary profile of optimal temperature $T_{stat}(t)$ in a batch process is a growing function of time with the final value of the optimal temperature

$$T_{opt}(t = t_f) = \min(T_{f,stat}, T_{max}) \quad (17)$$

where

$$T_{f,stat} = \left\{ \frac{R}{E_R} \ln \left[\frac{k_{P0} t_f}{E(1 - \bar{C}_{Sf}^{1/E})} \frac{\bar{C}_{Ef} (\bar{C}_{Sf})^{1/E}}{(k_{PM} + \bar{C}_{Ef})} \right] \right\}^{-1} \quad (18)$$

Now, taking into account eq. (15) in expression (5b) enables formulating a differential equation that describes an optimal varying activity with time $\bar{C}_{E,stat}(t)$

$$\frac{d\bar{C}_E}{dt} = -\frac{k_{D0}}{\bar{K}_D} k_{PM} \left[\frac{E(1-\bar{C}_{Sf}^{1/E})}{k_{P0} t_f} \right]^E \left(\frac{k_{PM} + \bar{C}_E}{\bar{C}_E} \right)^{(E-1)} \quad (19)$$

This equation can be solved numerically, since in view of eqs. (15) and (16), it significantly simplifies the overall computational procedure. However, for the integer values of the quotient of the activation energy $E = E_D/E_R$ it is possible to integrate analytically eq. (19) and obtain the following relations

$$\frac{k_{DM}}{k_{P0}^{(E-1)} \bar{K}_D} \left[\frac{E(1-\bar{C}_{Sf}^{1/E})}{t_f} \right]^E t = M(\bar{C}_{E0}) - M(\bar{C}_{E,stat}) \quad (20a)$$

$$M(\bar{C}_E) = \bar{C}_E - k_{PM} \left[(E-1) \ln(k_{PM} + \bar{C}_E) - \sum_{j=2}^{E-1} (-1)^{(j)} \frac{\binom{E-1}{j}}{(j-1)} \left(\frac{k_{PM}}{k_{PM} + \bar{C}_E} \right)^{(j-1)} \right] \quad (20b)$$

$(j = 2, 3, 4, 5, \dots, E-1)$

where

$$k_{DM} = k_{D0} K_M / (k_{R0} C_{E0})$$

and the functions $M(\bar{C}_{E0})$ as well as $M(\bar{C}_{E,stat})$ are expressed by eq. (20b) for $\bar{C}_E = \bar{C}_{E0} = 1$ and $\bar{C}_E = \bar{C}_{E,stat}$, respectively.

The duration time of the process $t_{f,stat}$ ($t_{f,stat} = t_f$) that is searched in the analyzed optimization problem results from the condition for the final state of biocatalyst $\bar{C}_{E,stat}(t = t_f) = \bar{C}_{Ef}$ and is given by the following equation ($\bar{C}_{E0} = 1$):

$$t_{f,stat} = k_{P0}^{-1} \left\{ \frac{k_{DM}}{\bar{K}_D} \frac{[E(1-\bar{C}_{Sf}^{1/E})]^E}{M(\bar{C}_{E0}) - M(\bar{C}_{Ef})} \right\}^{1/(E-1)} \quad (21)$$

where the function $M(\bar{C}_{Ef})$ is expressed by eq. (20b) for $\bar{C}_E = \bar{C}_{Ef}$.

More accurate analysis has revealed⁴⁹ that a decisive effect on the structure of the optimal temperature profile is exerted by the activation energy quotient E and a relation between the levels of the allowable temperatures T_{min} and T_{max} , the initial T_0 (eq. (14)) and final $T_{f,stat}$ (eq. (18)) temperature of the stationary profile of optimal temperature as well as the value of the isothermal process temperature T_{isot} (eq. (7)) resulting from its limiting values.

If $T_{min} \leq T_0$ and $T_{max} \geq T_{f,stat}$ then the optimal temperature profile is entirely stationary, described by eq. (15) and expressions (16), (20) and (21). If

one of these conditions is not fulfilled, it may activate a corresponding constraint.

If the permissible temperatures obey the relations

$$T_0 < T_{min} < T_{isot}$$

$$T_{isot} < T_{max} < T_{f,stat}$$

then, in an optimal process, the following elements will subsequently appear: an isothermal part at a level $T = T_{min}$, a nonisothermal part $T_{stat}(t)$, and an isothermal part at a level $T = T_{max}$ (the most frequent case).

If finally $T_{max} \leq T_{isot}$ or $T_{min} \geq T_{isot}$ the optimal policy is strictly isothermal.

The points at which transition of the lower isothermal part $T = T_{min}$ into a nonisothermal one $T_{stat}(t)$ and the latter into the upper isothermal one $T = T_{max}$ takes place, can be found from the following equation

$$\exp \left[\frac{E_R}{R} \left(\frac{1}{T_{min}} - \frac{1}{T_{max}} \right) \right] = \quad (22)$$

$$= \frac{\bar{C}_{E,b1}}{\bar{C}_{E,b2}} \left(\frac{k_{PM} + \bar{C}_{E,b2}}{k_{PM} + \bar{C}_{E,b1}} \right) \left[\frac{1 - \vartheta(T_{min})(1 - \bar{C}_{E,b1})}{\bar{C}_{Sf} - \vartheta(T_{max})(\bar{C}_{Ef} - \bar{C}_{E,b2})} \right]^{1/E}$$

where $\vartheta(T_{min})$ and $\vartheta(T_{max})$ are described in the same way as in eq. (6) for $T = T_{min}$ and $T = T_{max}$, respectively. Furthermore, $\bar{C}_{E,b1}$, $\bar{C}_{S,b1}$ and $\bar{C}_{E,b2}$, $\bar{C}_{S,b2}$ denote dimensionless biocatalyst activity and substrate concentration related to the end of the lower ($T = T_{min}$) and the beginning of the upper end ($T = T_{max}$) of the temperature constraint, respectively.

Transferring all terms in eq. (22) to one side gives in effect a two-variable function $\Psi(\bar{C}_{E,b1}, \bar{C}_{E,b2})$, which minimum value determines the searched point $(\bar{C}_{E,b1}, \bar{C}_{E,b2})$.

The substrate concentration $\bar{C}_{S,b1}$ and $\bar{C}_{S,b2}$ corresponding to the established values of the biocatalyst activity $\bar{C}_{E,b1}$ and $\bar{C}_{E,b2}$ are obtained from eq. (6).

It should be noticed that in case of $T_{max} \leq T_{isot}$ or $T_{min} \geq T_{isot}$ the optimal temperature profile does not ensure simultaneous achievement of the predetermined final values of \bar{C}_{Ef} and \bar{C}_{Sf} .

Comparison between isothermal and optimal temperature policy

Application of an optimal varying temperature $T_{stat}(t)$ enables a considerable reduction in the duration time of the batch process as compared to that for an identical process carried out under isothermal conditions T_{isot} .⁵⁰

In order to check how much more favourable it is to run the process at a stationary profile of optimal temperature in the situation considered in this paper, the same analysis has been performed by determining the value of the process time quotient $t_{f, \text{isot}}/t_{f, \text{opt}}$ under the analyzed temperature conditions. From the practical point of view such an approach, in the simplest and most appropriate way, justifies (or not) the application of the optimal temperature profile in the biotransformation processes described by the presented mathematical model (5) for the specified value of E , \bar{C}_{Ef} and \bar{C}_{Sf} .

The quotient results from division of eqs. (8) (for $E_p = E_R$) and (21) by sides for $\bar{C}_{\text{E0}} = 1$ and $\bar{C}_{\text{S0}} = 1$. This leads to the following expressions

$$\frac{t_{f, \text{isot}}}{t_{f, \text{stat}}} = \frac{k_{\text{PM}} \frac{(1 - \bar{C}_{\text{Ef}})}{\bar{C}_{\text{Ef}}} - \ln(\bar{C}_{\text{Ef}})}{\left\{ [E(1 - \bar{C}_{\text{Ef}}^{1/E})]^E \cdot [M(\bar{C}_{\text{E0}}) - M(\bar{C}_{\text{Ef}})]^{-1} \right\}^{1/(E-1)}} \quad (23a)$$

for $\bar{C}_{\text{Sf}} = \bar{C}_{\text{Ef}}$

$$\frac{t_{f, \text{isot}}}{t_{f, \text{stat}}} = \frac{k_{\text{PM}}(1 - \bar{C}_{\text{Sf}}) \frac{\ln(\bar{C}_{\text{Sf}}/\bar{C}_{\text{Ef}})}{(\bar{C}_{\text{Sf}} - \bar{C}_{\text{Ef}})} - \ln(\bar{C}_{\text{Sf}})}{\left\{ \frac{(1 - \bar{C}_{\text{Ef}})}{1 - \bar{C}_{\text{Sf}}} \cdot [E(1 - \bar{C}_{\text{Sf}}^{1/E})]^E \cdot [M(\bar{C}_{\text{E0}}) - M(\bar{C}_{\text{Ef}})]^{-1} \right\}^{1/(E-1)}} \quad (23b)$$

for $\bar{C}_{\text{Sf}} \neq \bar{C}_{\text{Ef}}$.

As it follows from eqs. (23), the quotient $t_{f, \text{isot}}/t_{f, \text{stat}}$ is dependent not only on parameter E and the final catalyst activity \bar{C}_{Ef} as it has taken place in the case of deactivation independent of the substrate concentration.⁴¹ It is dependent also on the value of \bar{C}_{Sf} and kinetic parameters (k_{PM}) describing the process under consideration.

The presence of the cell membrane is represented by the effectiveness factor η^{51-55} of the enzyme contained within the cells. This effectiveness factor is defined as the ratio of the reaction rate at substrate concentration inside a cell (observed reaction rate) to reaction rate at substrate concentration in a fluid core. Values of the coefficient of effectiveness $\eta \rightarrow 1$ ($k_{\text{PM}} \rightarrow \infty$) correspond to a situation that the biotransformation process undergoes in the presence of a native enzyme. Then eqs. (23) take the form

$$\frac{t_{f, \text{isot}}}{t_{f, \text{stat}}} = E^{-(E+1)/(E-1)} \left[\frac{(1 - \bar{C}_{\text{Ef}}^E)}{(1 - \bar{C}_{\text{Ef}}^{1/E})^E} \right]^{1/(E-1)} \frac{(1 - \bar{C}_{\text{Ef}})}{\bar{C}_{\text{Ef}}} \quad (24a)$$

for $\bar{C}_{\text{Ef}} = \bar{C}_{\text{Sf}}$

$$\frac{t_{f, \text{isot}}}{t_{f, \text{stat}}} = E^{-(E+1)/(E-1)} \left[\frac{(1 - \bar{C}_{\text{Sf}})^E (1 - \bar{C}_{\text{Ef}}^E)}{(1 - \bar{C}_{\text{Sf}}^{1/E})^E (1 - \bar{C}_{\text{Ef}})} \right]^{1/(E-1)} \cdot \frac{\ln(\bar{C}_{\text{Ef}}/\bar{C}_{\text{Sf}})}{(\bar{C}_{\text{Ef}} - \bar{C}_{\text{Sf}})} \quad (24b)$$

for $\bar{C}_{\text{Ef}} \neq \bar{C}_{\text{Sf}}$.

Analysis of results

In order to illustrate the dependencies given above (eqs. (15), (23) and (24)), the experimental data collected from laboratory investigations on the decomposition process of hydrogen peroxide by catalase in the yeast cells *Saccharomyces cerevisiae* have been used (cf. Table 1, unpublished data).

Table 1 – Parameters used in calculation

Parameter	Value
C_{S0}	0.01 kmol m ⁻³
E_R	4900 J mol ⁻¹
E_D	61700 J mol ⁻¹
E_p	74300 J mol ⁻¹
$k_R C_{\text{E0}}$	$0.3 \cdot 10^{-4}$ kmol ² m ⁻⁶ s ⁻¹
k_D	$1.92 \cdot 10^{-4}$ s ⁻¹
K_D	0.016 kmol m ⁻³
K_M	0.083 kmol m ⁻³

For the value of E_R , the computations have been expanded by adding the values of the activation energy of the deactivation process E_D for which the quotient $E = E_D/E_R$ is within the limits from 2 to about 13. The values of this quotient E refer to the majority of the processes that are encountered in industrial practice⁴¹ thus providing a generalization for the range of the present considerations.

The coefficients of frequency k_{D0} , corresponding to the assumed values of E_D in calculations, have been obtained based on the half-time decrease of biocatalyst activity, determined from the kinetic data and accounted in the solution of the mathematical model (5).

When analyzing the optimal temperature profiles, the lower and upper temperature limits have additionally been accounted for at the values $T_{\min} = 293$ K and $T_{\max} = 323$ K.

Phase diagram for isothermal process

In order to illustrate the temperature effect of the isothermal process on the course of trajectory $\bar{C}_E = \bar{C}_E(\bar{C}_S)$ in the state space, a phase diagram has been prepared (Fig. 1).

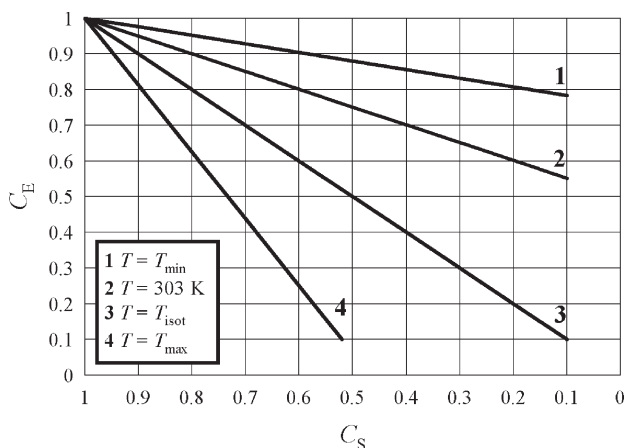


Fig. 1 – Phase diagram for isothermal condition and activation energy quotient $E = 12$

From this diagram it is concluded that there exists only a single value of temperature (eq. (7)) ensuring running the process to the fixed final point ($\bar{C}_{Sf} = 0.1$, $\bar{C}_{Ef} = 0.1$). Values of temperature, T , at which $T < T_{\text{isot}}$ result in an increase of the reaction rate. The temperatures $T > T_{\text{isot}}$ are preferable for the process of catalase deactivation.

The course of the optimal temperature profiles

For the selected values of quotient E the optimal temperature profiles are shown in Fig. 2. They were obtained using the relative time $\tau = t/t_f$.

In contrast to processes with (bio-)catalyst deactivation independent of substrate concentration⁴² the optimal temperature profiles for processes with parallel deactivation usually contain at least the upper temperature constraint $T = T_{\max}$. It should be pointed out, however, that at low values of the parameter E , the optimal temperature profiles assume the range of its greater values. As a result, at such values of E and at the active temperature constraints, the optimal temperature policy is usually strictly isothermal at the level $T = T_{\max}$.

As it has already been mentioned, the presence of the cell membrane is represented by the effectiveness factor η of enzyme contained within the cells. Its impact on the optimal temperature profile is de-

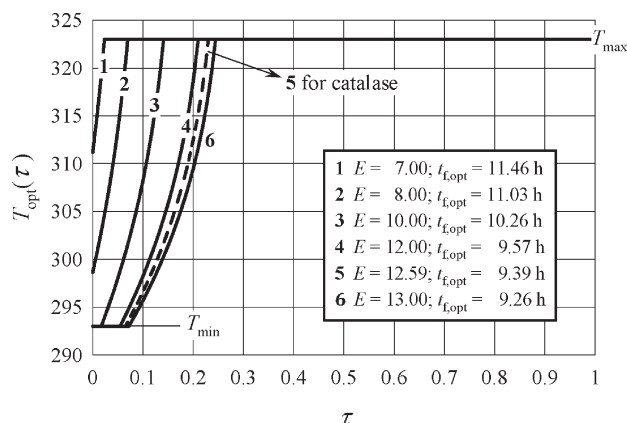


Fig. 2 – Effect of the quotient of the activation energy E on the optimal temperature profiles for the effectiveness factor $\eta = 0.5$, the final values of biocatalyst activity and substrate concentration equal to $\bar{C}_{Ef} = 0.1$ and $\bar{C}_{Sf} = 0.1$, respectively, as well as for the activation energy for permeability of the cell membrane $E_p = E_R$

picted in Fig. 3. Based on its value (η) the frequency factor for permeability of the cells membrane k_{p0} has been determined. A value of $\eta \rightarrow 1$ ($k_{pM} \rightarrow \infty$) corresponds to a case where the diffusional resistance does not affect the reaction rate.

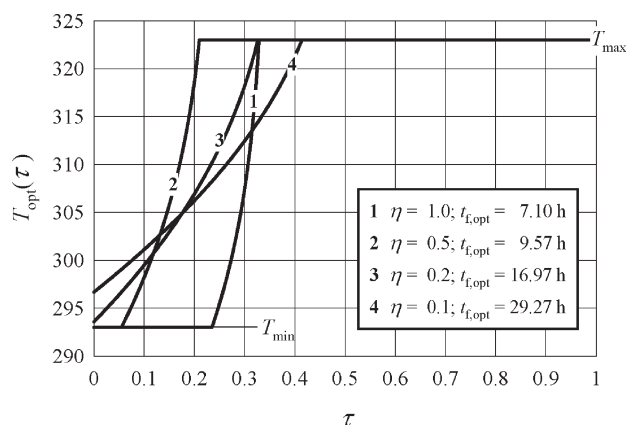


Fig. 3 – Effect of the effectiveness factor η on the optimal temperature profiles for the quotient of the activation energy $E = 12$, the activation energy for permeability of the cells membrane $E_p = E_R$ as well as the final values of biocatalyst activity and substrate concentration equal to $\bar{C}_{Ef} = 0.1$ and $\bar{C}_{Sf} = 0.1$, respectively

In addition, in Figs. 4–6 the course of the trajectory $\bar{C}_{E,\text{opt}} = \bar{C}_E(\bar{C}_{S,\text{opt}})$ was plotted under the optimal conditions for the selected values of the parameters E and η with $E_p = E_R$.

As it follows from these graphs, the higher the final values of \bar{C}_{Ef} and \bar{C}_{Sf} , the shorter is the time of process completion. Lower values of the quotient of activation energy and the coefficient of biocatalyst effectiveness cause the optimal temperature conditions to tend toward isothermal conditions. This case is shown in Fig. 5 (line 4).

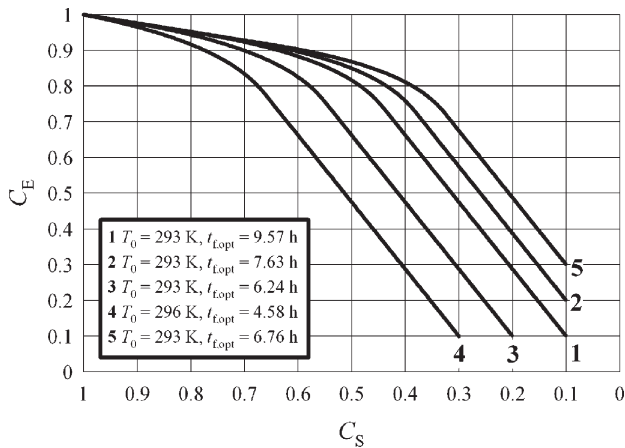


Fig. 4 – Phase diagram for optimal conditions as well as activation energy quotient $E = 12$ and $\eta = 0.5$

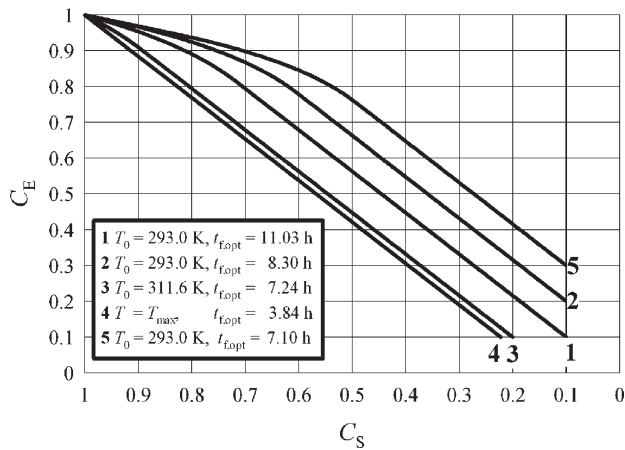


Fig. 5 – Phase diagram for optimal conditions as well as activation energy quotient $E = 8$ and $\eta = 0.5$

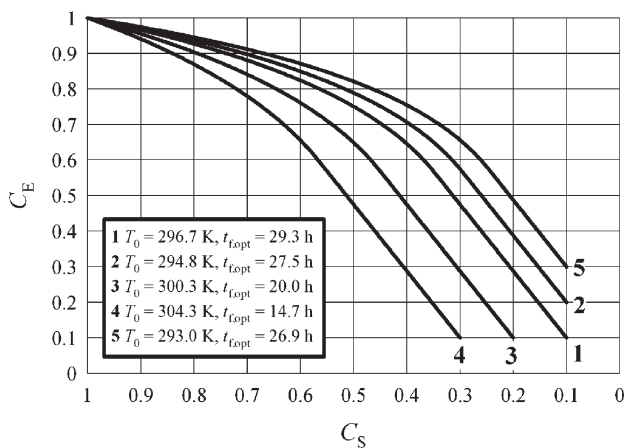


Fig. 6 – Phase diagram for optimal conditions as well as activation energy quotient $E = 12$ and $\eta = 0.1$

The lower values of the quotient of the activation energy and the coefficient of biocatalyst effectiveness result in the tendency of the optimal temperature conditions toward isothermal ones. This case is indicated in Fig. 5 (line 4), where the final

values of \bar{C}_{Ef} and \bar{C}_{Sf} equal 0.1 and 0.3, respectively, have been assumed. Under such conditions the temperature of the isothermal process $T_{\text{isot}} > T_{\text{max}}$, so the optimal policy is strictly isothermal at a level $T = T_{\text{max}}$. It is then not possible to simultaneously achieve the fixed values of \bar{C}_{Ef} and \bar{C}_{Sf} . Namely, for $\bar{C}_{\text{Sf}} = 0.3$ the final activity amounts to $\bar{C}_{\text{Ef}} = 0.19$, while at $\bar{C}_{\text{Ef}} = 0.1$ the final substrate concentration is $\bar{C}_{\text{Sf}} = 0.3$.

Since temperature significantly affects transfer processes across the cell wall, the courses of the optimal temperature profiles $T_{\text{opt}}(\tau)$ at different values of the activation energy E_p of the transfer process across the cell wall are displayed in Figs. 7 and 8.

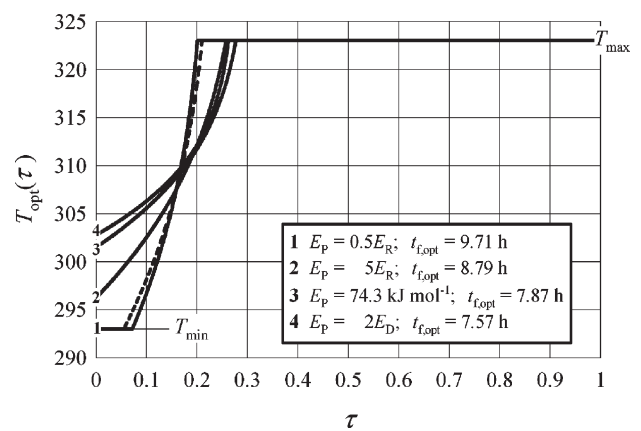


Fig. 7 – Effect of the activation energy for permeability of the cell membrane E_p on the optimal temperature profiles for the quotient of the activation energy $E = 12$, a value of the effectiveness factor $\eta = 0.5$ as well as the final values of biocatalyst activity and substrate concentration equal to $\bar{C}_{\text{Ef}} = 0.1$ and $\bar{C}_{\text{Sf}} = 0.1$, respectively. Dashed line in this figure determines the optimal temperature profile for $E_p = E_R$. The remaining profiles have been obtained from numerical computations.

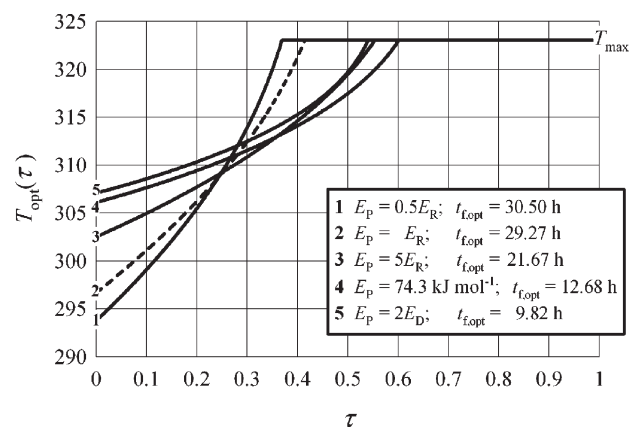


Fig. 8 – Effect of the activation energy for permeability of the cell membrane E_p on the optimal temperature profiles for the quotient of the activation energy $E = 12$, a value of the effectiveness factor $\eta = 0.1$ as well as the final values of biocatalyst activity and substrate concentration equal to $\bar{C}_{\text{Ef}} = 0.1$ and $\bar{C}_{\text{Sf}} = 0.1$, respectively. Dashed line in this figure determines the optimal temperature profile for $E_p = E_R$. The remaining profiles have been obtained from numerical computations.

The dashed lines in these figures determine the optimal temperature profile for $E_p = E_R$. The remaining profiles were obtained from numerical computations.

As it follows from Figs. 7 and 8, the temperature profiles are located at higher values for increasing values of E_p . In consequence the lower constraint becomes inactive and the total process duration time $t_{f,opt}$ decreases.

The process analyzed in the work is of great practical importance^{56,57} and usually is carried out under isothermal conditions (the simplest for application of the temperature conditions) at temperatures above 60 °C. At the same time overlooked is the fact that the optimal activity of catalase, similarly as for the majority of enzymes, is achieved in the range of moderate temperatures (20 °C – 50 °C).⁵⁸ Thus, the temperature profiles given above (Figs. (2) and (3) as well as (7) and (8)) can be used as an approximation of the real optimal temperature conditions.

Optimal temperature profiles accompanying biotransformation processes frequently hold in their structure at least an upper temperature constraint. However, it is worth mentioning that in the case of processes with deactivating biocatalyst independent of the substrate concentration, the optimal temperature profiles may not be constrained by permissible temperatures $T = T_{min}$ and $T = T_{max}$, and usually are a monotonically increasing time function.⁵⁹ In particular, this becomes evident for $E_D > E_p$. However, at $E_D \leq E_p$ and low values of biocatalyst effectiveness ($\eta \leq 0.2$) a course of a stationary optimal temperature uncommon for enzymatic processes is seen. Actually, at slightly higher final activities, e.g. $\bar{C}_{Ef} = 0.3$, one can notice an appearance of a temperature minimum ($\eta = 0.2$) or its decrease ($\eta = 0.1$) taking place for the entire process duration time.

A decrease in the final biocatalyst activity ($\bar{C}_{Ef} = 0.1$) results in the course of the temperature profiles within the range of higher values, giving rise to a situation that a temperature minimum becomes visible. It moves towards the end of the process as η decreases.

Results of the comparative analysis

Application of a optimal varying temperature usually results in a significant reduction in process duration time $t_{f,stat}$ as compared to that when the process is conducted under isothermal conditions $t_{f,isot}$. Equations (23) and (24) enable to determine the magnitude of these time savings. Their graphical interpretation for different process parameters is depicted in Figs. 9–11.

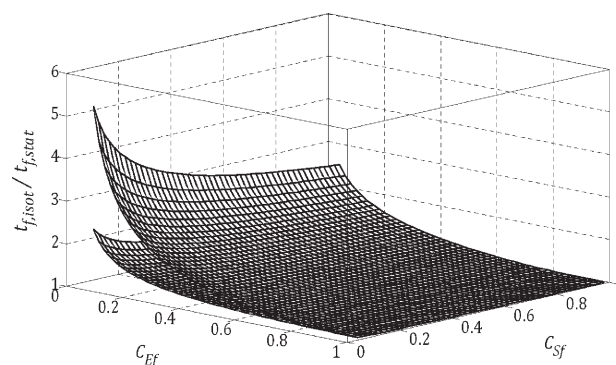


Fig. 9 – Effect of the final values of biocatalyst activity \bar{C}_{Ef} and substrate concentration C_{Sf} as well as cell membrane permeability (η) on the values of the quotient $t_{f,isot}/t_{f,stat}$ for parameter $E = 12.0$. The upper plane depicts values of the analyzed quotient for native enzyme ($\eta = 1$), while the lower one for microorganism cells at the effectiveness factor $\eta = 0.3$.

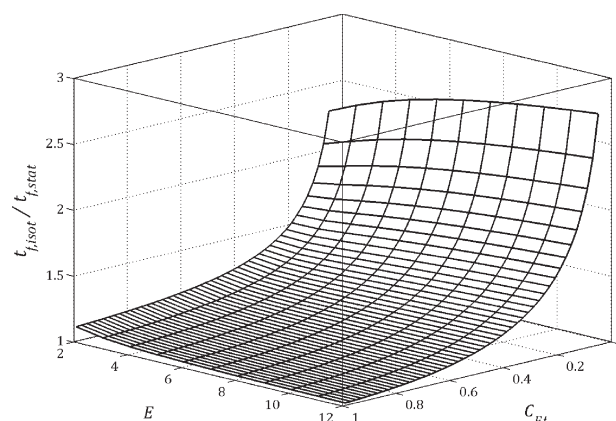


Fig. 10 – Effect of the activation energy quotient E and the final activity of biocatalyst \bar{C}_{Ef} on the quotient $t_{f,isot}/t_{f,stat}$ for the final substrate concentration $C_{Sf} = 0.1$ and the effectiveness factor $\eta = 0.5$

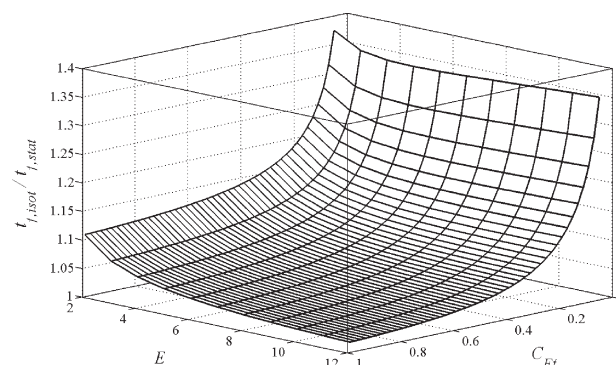


Fig. 11 – Effect of the activation energy quotient E and the final activity of biocatalyst \bar{C}_{Ef} on the quotient $t_{f,isot}/t_{f,stat}$ for the final substrate concentration $C_{Sf} = 0.1$ and the effectiveness factor $\eta = 0.1$

Now, making use of the stationary profile of optimal temperature, the most significant cut down in process duration time (increase in conver-

sion $\alpha_f = 1 - \bar{C}_{Sf}$) compared with its course under isothermal conditions is obtained by conducting the process until achieving possibly low final substrate concentration \bar{C}_{Sf} and biocatalyst activity \bar{C}_{Ef} (Fig. 9).

The mentioned advantage will greatly be increased with increasing values of the activation energy quotient E (Fig. 10).

The presence of the cell membrane lowers values of the quotient $t_{f, \text{isot}}/t_{f, \text{stat}}$ under the analyzed temperature conditions. At the same time it decreases with diminishing values of the coefficient of effectiveness η (permeation of cell membrane). It should be pointed out that slightly higher values of \bar{C}_{Ef} and too low permeability of cell membrane (η) result in a deviation from the above-discussed regularity (Fig. 11).

It is seen that with an increasing quotient of the activation energy E a minimum of the quotient $t_{f, \text{isot}}/t_{f, \text{stat}}$ or its decline depending on the values of \bar{C}_{Ef} and η are observed.

Conclusions

An analysis aiming at searching for an optimal temperature policy ensuring the shortest duration time of a biotransformation process with a parallel deactivation of enzyme encapsulated inside the microorganism cells has been presented. A batch process running within the range of low values of the concentrations inside the cells ($C_{S \text{in}} \ll K_D, C_{S \text{in}} \ll K_M$) under isothermal conditions and at a stationary profile of the optimal temperature has been considered.

A case, for which there exists an analytical solution to the problem under consideration has been indicated. On this basis, the requirements necessary to conduct the process under the analyzed temperature conditions determined. Though this refers to a special case ($E_p = E_R$), it still emphasizes all regularities that exist for the processes running in the presence of microorganism cells.

It has been proved that for processes with catalyst deactivation, both parallel as well as independent of the substrate concentration, the temperature profile is an increasing function of time. Additionally, in the case of parallel deactivation there is usually a requirement to account for at least the upper temperature constraint $T = T_{\text{max}}$.

Application of microorganism cells results in slowing down the reaction rate and shifting the initial temperature of the stationary profile to higher values. They are more pronounced

the lower the permeability of the cell membrane (η). Consequently, an extension of the process duration time is observed along the sections of the optimal profile while the lower temperature constraint usually becomes inactive. Furthermore, lowering values of parameter E causes the optimal temperature profiles to shift towards higher values. In effect, the optimal policy consists of the following subsequent parts: isothermal $T = T_{\text{min}}$, stationary $T_{\text{stat}}(t)$ and isothermal $T = T_{\text{max}}$, at high values of E , throughout the profiles with an inactive lower temperature constraint, or becomes strictly an isothermal policy at relatively low values of E .

For the case analyzed in this work ($E_p = E_R$) and the situation where application of the temperature constraints is not required, the advantages resulting from applying an optimal varying temperature with respect to the strictly isothermal policy have been assessed. These were expressed by a quotient of process duration times $t_{f, \text{isot}}/t_{f, \text{stat}}$ under the analyzed temperature conditions (eqs. (23)).

From the obtained relationship its equivalent (eqs. (24)) for the case when using a native enzyme in biocatalysis ($\eta = 1$) has been determined.

The computations have revealed that the resulting quotient $t_{f, \text{isot}}/t_{f, \text{stat}}$ is dependent not only on the activation energy quotient E and the final activity of biocatalyst \bar{C}_{Ef} , as it was the case for processes with catalyst inactivation independent of the substrate concentration. Additionally, it is dependent on the value of \bar{C}_{Sf} while using microorganism cells and also on the effectiveness factor of biocatalyst $k_{PM}(\eta)$.

In order to attain the fixed final values of the state variables \bar{C}_{Ef} and \bar{C}_{Sf} , application of the stationary profile of optimal temperature in the process with parallel inactivation results in a much shorter duration time of the process $t_{f, \text{stat}}$ with respect to the isothermal conditions $t_{f, \text{isot}}$, as compared to an analogue process with deactivation independent on the substrate concentration.

In addition, the quotient of the mentioned duration times increases with increasing values of E as well as decreasing values of \bar{C}_{Ef} and \bar{C}_{Sf} . Diminishing permeability (η) of the cell membrane causes a decrease of difference between the initial and the final temperatures of the non-isothermal profile and results in making the temperature conditions tend toward the isothermal conditions.

Appendix A.

Derivation of the equation for stationary optimal decision using variational method

Let

$$\frac{d\bar{C}_S}{dt} = f(t, \bar{C}_S, \bar{C}_E, \Theta) \quad (\text{A.1})$$

$$\frac{d\bar{C}_E}{dt} = g(t, \bar{C}_S, \bar{C}_E, \Theta) \quad (\text{A.2})$$

determine state equations in an optimization problem minimizing the process duration time. Now, the objective function takes the following form

$$S = \int_0^{t_f} dt \quad (\text{A.3})$$

which is a special case of the Bolza functional and determines the so-called Lagrange problem.

Since the mathematical model (eqs. (A.1) and (A.2)) is dependent on the two state variables (\bar{C}_E, \bar{C}_S) and one decisive variable (Θ), then when solving the problem, it is possible to determine a differential equation that describes a stationary control.

For this purpose, by introducing the Lagrange multipliers $\lambda_i(t)$ ($i = E, S$) it is indispensable to formulate a modified objective function S_R defined as

$$S_R = \int_0^{t_f} \left\{ 1 + \lambda_S \left[\frac{d\bar{C}_S}{dt} - f(t, \bar{C}_S, \bar{C}_E, \Theta) \right] + \lambda_E \left[\frac{d\bar{C}_E}{dt} - g(t, \bar{C}_S, \bar{C}_E, \Theta) \right] \right\} dt \quad (\text{A.4})$$

which will undergo minimization procedure. The functions $\bar{C}_{E, \text{stat}}(t)$ and $\bar{C}_{S, \text{stat}}(t)$ that minimize this functional are sought as the solutions of the Euler-Lagrange equations being the necessary⁴⁸ condition for the existence of the functional extreme (A.4)

$$\frac{d}{dt} \left[\frac{\partial L}{\partial (dy/dt)} \right] - \frac{\partial L}{\partial y} = 0 \quad (y = \bar{C}_E, \bar{C}_S, \Theta) \quad (\text{A.5})$$

where $L(\bar{C}_E, \bar{C}_S, \Theta, d\bar{C}_E/dt, d\bar{C}_S/dt)$ is a function expressed in the form of

$$L = 1 + \lambda_S \left[\frac{d\bar{C}_S}{dt} - f(t, \bar{C}_S, \bar{C}_E, \Theta) \right] + \lambda_E \left[\frac{d\bar{C}_E}{dt} - g(t, \bar{C}_S, \bar{C}_E, \Theta) \right] \quad (\text{A.6})$$

Calculating the variables that appear in equations (A.5) yields

$$\frac{d\lambda_S}{dt} = -\lambda_S \frac{\partial f(t, \bar{C}_E, \bar{C}_S, \Theta)}{\partial \bar{C}_S} - \lambda_E \frac{\partial g(t, \bar{C}_E, \bar{C}_S, \Theta)}{\partial \bar{C}_S} \quad (\text{A.7})$$

$$\frac{d\lambda_E}{dt} = -\lambda_S \frac{\partial f(t, \bar{C}_E, \bar{C}_S, \Theta)}{\partial \bar{C}_E} - \lambda_E \frac{\partial g(t, \bar{C}_E, \bar{C}_S, \Theta)}{\partial \bar{C}_E} \quad (\text{A.8})$$

$$-\lambda_S \frac{\partial f(t, \bar{C}_E, \bar{C}_S, \Theta)}{\partial \Theta} - \lambda_E \frac{\partial g(t, \bar{C}_E, \bar{C}_S, \Theta)}{\partial \Theta} = 0 \quad (\text{A.9})$$

From the above set of equations (A.7) – (A.9) it is possible to eliminate the variables $\lambda_E(t)$ and $\lambda_S(t)$. Assessing the multiplier λ_E from eq. (A.9)

$$\lambda_E = -\lambda_S \frac{\partial f(t, \bar{C}_E, \bar{C}_S, \Theta)}{\partial g(t, \bar{C}_E, \bar{C}_S, \Theta)} \quad (\text{A.10})$$

and accounting for its value in eq. (A.8), then dividing it side by side by λ_S , one can obtain (for clarity of the notation in some of the expressions in the further part of this derivation it is assumed $f(t, \bar{C}_E, \bar{C}_S, \Theta) = f$, $g(t, \bar{C}_E, \bar{C}_S, \Theta) = g$, $Z_H(t, \bar{C}_E, \bar{C}_S, \Theta) = Z_H$)

$$\frac{dZ_H}{dt} = \frac{\partial f}{\partial \bar{C}_E} + Z_H \left(\frac{\partial f}{\partial \bar{C}_S} - \frac{\partial g}{\partial \bar{C}_E} \right) - Z_H^2 \frac{\partial g}{\partial \bar{C}_S} \quad (\text{A.11})$$

with

$$Z_H = \frac{\partial f}{\partial \Theta} / \frac{\partial g}{\partial \Theta} \quad (\text{A.12})$$

and is called the Horn variable.

Making use of a definition of the complete derivative Z_H with respect to time t , a searched equation describing changes of the stationary decision Θ is gained in time

$$\frac{d\Theta}{dt} = \left[\frac{\partial f}{\partial \bar{C}_E} + Z_H \left(\frac{\partial f}{\partial \bar{C}_S} - \frac{\partial g}{\partial \bar{C}_E} \right) - Z_H^2 \frac{\partial g}{\partial \bar{C}_S} - \frac{\partial Z_H}{\partial \bar{C}_S} f - \frac{\partial Z_H}{\partial \bar{C}_E} g - \frac{\partial Z_H}{\partial t} \right] \cdot \left(\frac{\partial Z_H}{\partial \Theta} \right)^{-1} \quad (\text{A.13})$$

Introducing additional notations

$$X_T = \frac{k'_R}{k_p K_M} \bar{C}_E \quad (\text{A.14})$$

the functions f and g in the state equation (5) can be transformed into

$$f(\bar{C}_E, \bar{C}_S, \Theta) = -\frac{(k'_R / \bar{K}_M) \bar{C}_E \bar{C}_S}{1 + X_T} \quad (\text{A.15})$$

$$g(\bar{C}_E, \bar{C}_S, \Theta) = -\frac{(k_D / \bar{K}_D) \bar{C}_E \bar{C}_S}{1 + X_T} \quad (\text{A.16})$$

while the Horn variable Z_H is given by

$$\frac{\partial f}{\partial \Theta} = \frac{(k'_R / \bar{K}_M) \bar{C}_E \bar{C}_S}{R(1 + X_T)} \left[E_R + (E_P - E_R) \frac{X_T}{(1 + X_T)} \right] \quad (\text{A.17})$$

$$\frac{\partial g}{\partial \Theta} = \frac{(k_D / \bar{K}_D) \bar{C}_E \bar{C}_S}{R(1 + X_T)} \left[E_D + (E_P - E_R) \frac{X_T}{(1 + X_T)} \right] \quad (\text{A.18})$$

$$Z_H = \frac{(\partial f / \partial \Theta)}{(\partial g / \partial \Theta)} = \frac{(k'_R / \bar{K}_M)}{(k_D / \bar{K}_D)} \frac{(E_R + E_P X_T)}{[E_D + (E_D + E_P - E_R) X_T]} \quad (\text{A.19})$$

where

$$\Theta = T^{-1}$$

$$k'_R = \frac{k_R C_{E0}}{C_{S0}}$$

After calculating the remaining variables in eq. (A.13) we obtain

$$\frac{\partial f}{\partial \bar{C}_E} = -\frac{k'_R}{\bar{K}_M} \frac{\bar{C}_S}{(1 + X_T)^2} \quad (\text{A.20})$$

$$\frac{\partial f}{\partial \bar{C}_S} = -\frac{k'_R}{K_M} \frac{\bar{C}_E}{(1 + X_T)} \quad (\text{A.21})$$

$$\frac{\partial g}{\partial \bar{C}_E} = -\frac{k_D}{K_D} \frac{\bar{C}_S}{(1 + X_T)^2} \quad (\text{A.22})$$

$$\frac{\partial g}{\partial \bar{C}_S} = -\frac{k_D}{K_D} \frac{\bar{C}_E}{(1 + X_T)} \quad (\text{A.23})$$

$$\frac{\partial Z_H}{\partial \bar{C}_E} = \frac{[E_D E_P - E_R (E_D + E_P - E_R)]}{k_p (k_D / \bar{K}_D)} \cdot \left[\frac{k'_R / \bar{K}_M}{E_D + (E_D + E_P - E_R) X_T} \right]^2 \quad (\text{A.24})$$

$$\frac{\partial Z_H}{\partial C_S} = 0 \quad (\text{A.25})$$

$$\frac{\partial Z_H}{\partial \Theta} = \frac{(k'_R / \bar{K}_M)}{(k_D / \bar{K}_D)} \left\{ \frac{(E_D - E_R)}{R} \frac{(E_R + E_P X_T)}{[E_D + (E_D + E_P - E_R) X_T]} + \frac{(E_P - E_R)}{R} \frac{[E_D E_P - E_R (E_D + E_P - E_R)] X_T}{[E_D + (E_D + E_P - E_R) X_T]^2} \right\} \quad (\text{A.26})$$

$$\frac{\partial Z_H}{\partial t} = 0 \quad (\text{autonomous process}) \quad (\text{A.27})$$

Substituting these expressions into eq. (A.13) and transforming it to the real control variable T yields

$$\frac{dT}{dt} = T^2 \frac{R}{E_D E_R} [1 + X_T \cdot (b_1 + b_2 X_T)]^{-1} \cdot \left[E_D \frac{k_D}{K_D} \bar{C}_S + (E_R + E_P X_T) \frac{k'_R}{K_M} \bar{C}_E \right] \quad (\text{A.28})$$

where

$$b_1 = \frac{(E_D + E_P - E_R)(E_D E_R + E_D E_P - E_P E_R)}{E_D E_R (E_D - E_R)} - \frac{E_P}{(E_D - E_R)}$$

$$b_2 = \frac{E_P}{E_D E_R} (E_D + E_P - E_R)$$

Symbols

C_i – substrate concentration ($i = S$), substrate concentration inside microorganism cells ($i = S_{in}$), enzyme concentration ($i = E$), kmol m^{-3}

$\bar{C}_{i,b1}, \bar{C}_{i,b2}$ – dimensionless concentration of substrate ($i = S$) and biocatalyst activity ($i = E$) related to the end of the lower ($T = T_{\min}$) or the beginning of the upper end ($T = T_{\max}$) of the temperature constraint, respectively (–)

E_j – activation energy for reaction ($j = R$), deactivation ($j = D$), permeability of the cells membrane ($j = P$), J kmol^{-1}

$E = E_D / E_R$ – quotient of the activation energy, dimensionless

k_{j0} – frequency factor for reaction ($j = R$), deactivation ($j = D$), permeability of the cells membrane ($j = P$), s^{-1}

k_j – constant of reaction rate ($j = R$), deactivation ($j = D$), permeability of the cells membrane ($j = P$), s^{-1}

K_D – Michaelis-Menten constant for deactivation, kmol m^{-3}

$\bar{K}_D = K_D / C_{S0}$ – dimensionless Michaelis-Menten constant for deactivation

K_M – Michaelis-Menten constant for reaction, kmol m^{-3}

$\bar{K}_M = K_M / C_{S0}$ – dimensionless Michaelis-Menten constant for reaction

T – temperature, K

T_{\min}, T_{\max} – lower and upper temperature constraints

t – time, s

α – conversion

η – (bio-)catalyst effectiveness factor

$\tau = t / t_f$ – dimensionless time

Subscripts

0 – denotes initial condition

f – denotes final condition

isot – isothermal

opt – optimal

stat – stationary

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