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The effect of temperature on the growth of A. niger in solid state fermentation

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Abstract A kinetic model of solid state fermentation with temperature deactivation of microorganisms is presented. The experimental results of cultivation of Aspergillus niger on a mixture of wheat bran and beet pulp in temperature range from 26 °C to 40 °C were used to estimate the parameters of the model. The activation energies of growth, thermal deactivation and maintenance have been calculated.

List of symbols

C_{CX}	mol/g	proportionality coefficient
E_d	J/mol	energy of activation for thermal deactivation
E_g	J/mol	energy of activation for growth
J_{CO2}	mol/gh	carbon dioxide evolution rate
k_d	h^{-1}	thermal deactivation constant
k_g	h^{-1}	growth kinetic constant
k_x	h^{-1}	net growth constant
m	h^{-1}	maintenance coefficient
N_{CO2}	mol	amount of carbon dioxide
$N_{m,CO2}$	mol	maximum amount of carbon dioxide generated by growth
t	h	time
X	g	dry biomass weight
X_m	g	maximum biomass weight
X^*	–	dimensionless biomass weight
$X_{0,r}$	g	real mass of inoculum
$X_{0,a}$	g	apparent mass of inoculum
X_0^*		dimensionless apparent mass of inoculum
β	–	dimensionless maintenance coefficient

1 Introduction

Hydrolases are produced by submerged cultivation or by solid state cultivation using moistened cereals and agricultural wastes. As the microorganisms in a solid state fermentation grow under conditions closer to their natural habitats, they may be more capable of producing certain enzymes and metabolites which usually will not be produced or will be produced only with low yield in a submerged culture. Taking into consideration the simplicity of the cultivation equipment and lower expense for operation, wider application of this traditional method is expected with the advanced knowledge and approaches in biochemical engineering.

The heat generated during the microorganism growth is conducted through the solid medium. It produces the temperature gradients in the medium. As a result the growth of microorganisms in the solid state cultivation proceeds in non-isothermal conditions and is affected strongly by heat transport in the medium and heat exchange between the medium and air. The quantitative descriptions of the influence of nonuniform temperature on the growth of microorganisms is essential for modelling and optimizing of solid-state fermenters.

In the present work the influence of temperature on the growth of *Aspergillus niger* in the solid state is investigated. A mathematical model describing the kinetics of fungi growth, heat and carbon dioxide evolution is used to analyze the temperature effect.

2 Mathematical model of solid state state cultivation

The growth of microorganism in the solid state cultivation can be described by the logistic curve [1, 2]:

$$r_g = k_g X\left(1-\frac{X}{X_m}\right). \tag{1}$$

The parameter X_m is the maximum amount of biomass for the given conditions. The Arrhenius relationship is assumed to represent the temperature dependence of the growth rate constant k_g.

The rate of temperature deactivation of microorganisms may be written according to the first order kinetics [3]:

$$r_d = k_d X. \tag{2}$$

The deactivation constant k_d is also assumed to obey the Arrhenius relation.

As the result the kinetics of microbial growth in the solid state is described by the logistic curve:

$$\frac{dX}{dt} = k_X X\left(1-\frac{X}{X_m}\right), \tag{3}$$

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where the kinetic constant k_X depends on temperature:

$$k_X = k_g^o \exp\left(-\frac{E_g}{RT}\right) - k_g^o \exp\left(-\frac{E_d}{RT}\right). \quad (4)$$

The initial condition for Eq. (3) is:

$$X = X_{0,r}, \quad \text{for } 0 < t < t_0, \quad (5)$$

where t_0 is the time of lag-phase duration.

The Eq. (5) relates two variables: the lag-phase time and the mass of inoculum. It is very difficult to find these values independently. A new initial condition is therefore proposed:

$$X = X_{0,a} \quad \text{for } t = 0, \quad (6)$$

where $X_{0,a}$ is an apparent mass of inoculum, a model parameter depending both on the time of lag phase and on the mass of inoculum. $X_{0,a}$ is a mass of inoculum from which the mass $X_{0,r}$, is obtained after time t_0 if microorganism grow according to the logistic law. Fig. 1 presents the idea of the apparent inoculum.

The rate of carbon dioxide evolution is assumed to depend on two terms. The first term is growth related, the second is related to the maintenance processes:

$$J_{CO_2} = \frac{dN_{CO_2}}{dt} = C_{CX}\left(\frac{dX}{dt} + mX\right). \quad (7)$$

Combining Eqs. (3), (6), and (7) one obtains

$$J_{CO_2} = k_X N_{m,CO_2} X^* (1 + \beta - X^*), \quad (8)$$

where

$$X^* = \frac{X}{X_m} = \frac{1}{1 + \frac{1 - X_0^*}{X_0^*} e^{-k_X t}} \quad (9)$$

is dimensionless biomass obtained by integration of Eq. (3) with Eq. (6),

$$X_0^* = \frac{X_{0,a}}{X_m} \quad (10)$$

is dimensionless apparent mass of inoculum,

$$\beta = \frac{m}{k_X} \quad (11)$$

is dimensionless rate of endogenous metabolism, and

$$N_{m,CO_2} = C_{CX} X_m \quad (12)$$

describes the maximum amount of carbon dioxide generated by microbial growth only.

The model presented above contains four independent constants: k_X, β, $N_{m,CO2}$, X_0^*. These parameters may be estimated from the changes of carbon dioxide evolution rate during solid state cultivation.

3 Materials and methods

3.1 Microorganism

The microorganism used was Aspergillus niger K-14 from the collection of Institute of Biotechnology of the Agricultural and Food Industry, Warsaw, Poland.

3.2 Inoculum

Spores of A. niger incubated on wheat bran were suspended in sterile water containing 0,01% of a surface-active agent (Tween 80). The suspension, 20 cm^3, containing 10^6–10^7 conidia per cm^3, was added to 800 g of the solid medium as the inoculum.

3.3 Medium

Wheat bran with an addition of beet pulp (20%) was moistened and sterilized at 121 °C for 1 hour before inoculation. The water content after inoculation was 60%.

3.4 Culture equipment

Figure 2 shows the culture equipment. Cultivation was performed in four glass tubes. Each tube contained 30 g of wet medium and was supplied with moistened air. The air flow through a tube was 6.5 dm^3/h. All tubes were placed in the thermostatic bath.

Tubes were connected with carbon dioxide analyzer (ULTRAMAT, SIMENS) for continuous measurements of carbon dioxide evolution rate.

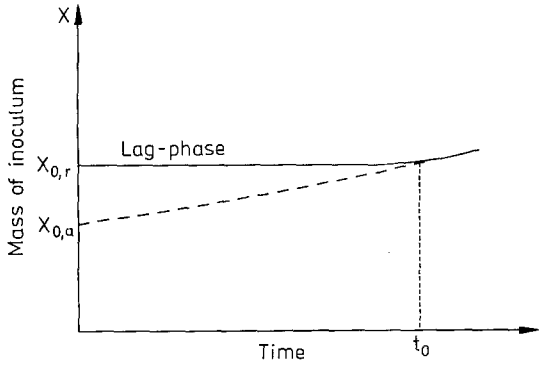

Fig. 1. Real and apparent mass of inoculum

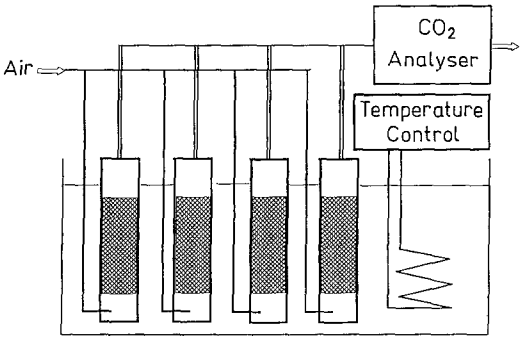

Fig. 2. Schematic diagram of the culture equipment

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Results and discussion

Figure 3 shows the changes of carbon dioxide evolution rate during cultivation at 28 °C and 34 °C. The solid lines present the model estimations. A good agreement between experimental results and model predictions is observed.

Figure 4 shows the temperature dependence of the kinetic constant k_X. The value of the constants increases with temperature for temperatures below 32 °C. It is the effect of the increase in k_g value. For temperatures above 32 °C, the value of k_X decreases as temperature increases. This is the effect of temperature deactivation. From estimated values of k_X the energies of activation and deactivation were calculated. The energy of activation for the growth process was estimated to be equal 46.5 kJ/mol. The energy of deactivation was found to be 219 kJ/mol. The last value agrees well with the known values of energy of activation for the thermal deactivation of various microorganisms: 200–400 kJ/mol [3].

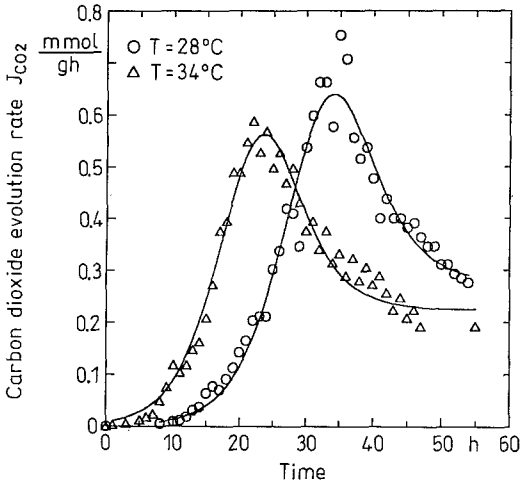

Fig. 3. The time changes of carbon dioxide evolution rate

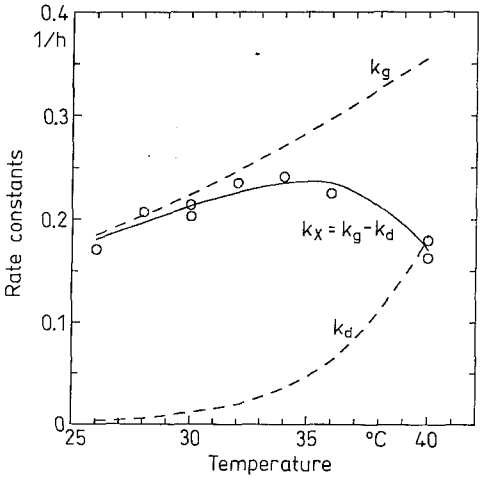

Fig. 4. The dependence of rate constants k_x, k_g, k_d on temperature

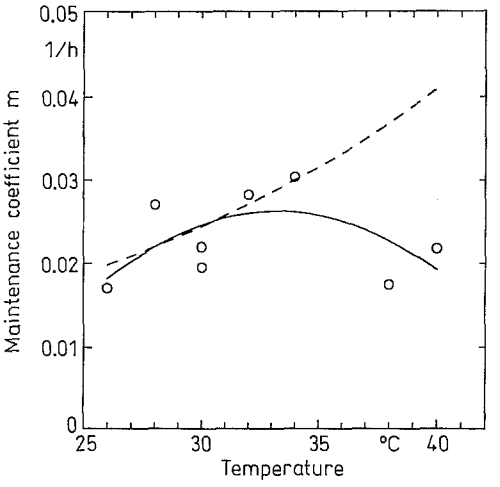

Fig. 5. The dependence of maintenance coefficient on temperature

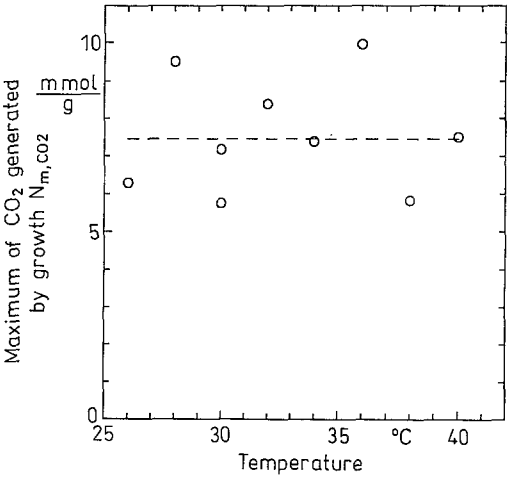

Fig. 6. The dependence of maximum amount of CO_2 generated by growth on temperature

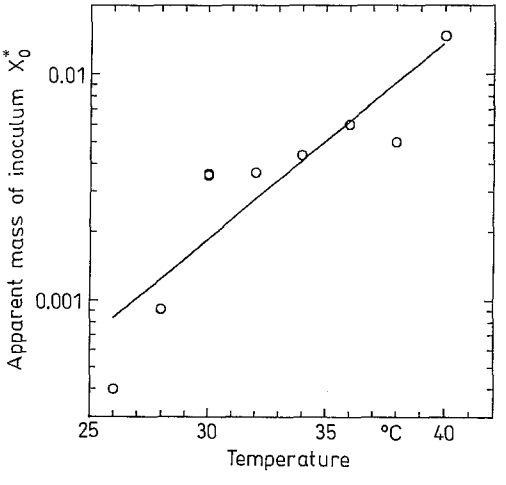

Fig. 7. The dependence of dimensionless apparent mass of inoculum on temperature

Figure 5 shows the dependence of endogenous metabolism coefficient on temperature. In the range of 26 to 34 °C the coefficient follows the Arrhenius relationship. The dashed line shows this relation. The energy of activation was found to be 40.5 kJ/mol. It agrees very well with the value 40 kJ/mol estimated by Heynen and Roels for submerged culture [4]. For temperature higher than 34 °C the maintenance coefficient decreases as temperature increases. This is a deactivation effect of temperature on endogenous metabolism. The solid line represents a nonlinear regression.

Figure 6 shows the dependence of the maximum amount of carbon dioxide evolved by growth ($N_{m,\,CO2}$) on temperature. It seems that this parameter is independent on the temperature. The average value is 7.45 mmol/g. The parameter depends on the space available for mold colonization and species characteristics.

Figure 7 presents the dependence of the apparent mass of inoculum on temperature. The apparent mass of inoculum increases as the lag phase period is getting shorter. The growth constant increases with temperature and, as a result, the lag phase period reduces as temperature increases. Fig. 7 shows that the apparent mass of inoculum increases with temperature.

5 Conclusions

The presented mathematical model of the growth of microorganisms in the solid state fermentation describes the non-isothermal conditions of cultivation. The effect of temperature deactivation is included in the well known logistic equation. Experimental results of the cultivation of Aspergillus niger on wheat bran and beet pulp agree very well with the model. The estimated values of energies of activation for growth and death processes agree well with the literature data for submerged cultures.

The results of experiments show that for the investigated microorganisms the temperature does not affect strongly the growth rate in the range of 28 °C–34 °C. This behavior is very favorable for stable cultivation in solid medium.

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