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Growth of the Thermotolerant Yeast, *Candida valida*, on Ethanol: Dependences of Maximal Growth Rate and Cell Biomass Yield on Temperature

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Summary. Growth of *Candida valida* on ethanol in pH-auxostat and chemostat has been studied. Maximal growth rate, μ_m, and cell biomass yield, Y_s, display the Arrhenius dependence on temperature within the ranges 18°–30° C and 30°–36° C and an abrupt fall above 36° C. The temperature dependence of both parameters has breaks at 30° C and 36° C. Activation energies have been measured for both μ_m and Y_s. The reason for a weaker effect of temperature on Y_s than on μ_m is discussed.

Introduction

Studies of the dependence of microbial growth characteristics on temperature are mostly aimed at obtaining a table or a graph showing that the parameter being studied has, for example, a maximum at a particular temperature. Such graphs are usually drawn by means of rough smoothing which thus tends to obscure the mechanism of the studied dependence. At the same time, there are a number of studies in which it has been found that the maximal growth rate, μ_m, over a wide temperature range fits the Arrhenius equation:

$$\mu_m = C_\mu e^{-\frac{E_\mu}{RT}} \quad (1)$$

where E_μ is the activation energy for μ_m, kJ · mol^{-1}; $T = 273 \pm t°$ C is the absolute temperature, K; R = 8.31 kJ · mol^{-1} · K^{-1} is the gas constant; C_μ is a constant (Farrell and Rose 1967; Topiwala and Sinclair 1971; Pirt 1975; Kuhn et al. 1980; Uchino and Katano 1981; Samoylenko and Petrikevich 1981; Reichardt and Morita 1982). The same has been shown for the parameters related to cell biomass yield (Kuhn et al. 1980). Arrhenius dependence in the field of microbial kinetics is in itself extremely important, since it allows the rigorous quantitative description of a temperature effect on a certain strain and the generalization of such data concerning many microorganisms, as well as facilitating an insight into the physico-chemical mechanisms of temperature dependence of microbial cells.

The number of micro-organisms for which the values of activation energy related to the rate and yield of growth have been obtained is not large, the organisms being mostly bacteria. We are not familiar with such data on thermotolerant yeasts. On the other hand, these yeasts are of interest from the viewpoint of SCP production, and cultivation at elevated temperature would make it possible to reduce fermentor cooling cost and can be expected to facilitate the control of infection. We have therefore studied the temperature dependence of maximal growth rate and the cell biomass yield of the thermotolerant yeast, *Candida valida*, during continuous cultivation on ethanol.

Material and Methods

Micro-Organism. Thermotolerant yeast *Candida valida* VKM-Y-2327 was obtained from the All-Union Culture Collection.

Media and Cultivation Conditions. Media were as described by Nguen et al. (1982).

Maximal growth rate was studied in pH-auxostat (Pechurkin et al. 1969; Martin and Hempfling 1976) which is a cultivation regime of a turbidostat type, but much more stable than the turbidostat. In pH-auxostat, the cultivation medium fed into a fermentor serves also as a titrant solution for the maintenance of a preset pH value in the fermentor. For this purpose, the the value of pH in the medium should be higher than the preset one in the fermentor. To avoid precipitation of mineral salts, the medium was prepared as four different solutions which were fed simultaneously into the fermentor; the rate of feed of each was determined by the

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difference between the preset and actual pH values in the fermentor. Stability of both biomass concentration and dilution rate in pH-auxostat is ensured by a very rapid response of pH in broth to small deviations from the regime. The value of cell biomass concentration is determined by the amount of ammonia consumed by cells from the medium, and the latter amount depends on the difference between the pH of the complete medium and the preset pH in the fermentor as well as on the buffer capacity of the complete medium.

Cell biomass yield was studied in ethanol-limited chemostat. Dilution rate at any temperature was established equal to one-third of μ_m measured at the same temperature in pH-auxostat. This ensured a low concentration of residual ethanol and a low rate of its evaporation. Foam formation was prevented by a mechanical foam breaker.

All experiments were conducted at pH = 4.0, $pO_2 \approx 60\%$.

Mathematical Description and Data Processing. The dependence of μ_m on temperature was searched for in the form of Eq. (1). The dependence of cell biomass yield Y_s (g/g) on temperature was searched for as follows:

$$Y_s = C_y e^{-\frac{E_Y}{RT}} \tag{2}$$

where E_Y is the activation energy for Y_s, kJ · mol^{-1}; C_Y is a constant. To find E and C, the plots of ln μ_m and ln Y_s[1] versus $1/T$ were drawn and linear parts of the plots were found visually and then approximated by the following linear functions:

$$\ln \mu_m = \ln C_\mu - \frac{E_\mu}{R}\frac{1}{T} \tag{3}$$

$$\ln Y_s = \ln C_Y - \frac{E_Y}{R}\frac{1}{T} \tag{4}$$

using the least-square technique. Regions of sharp fall of μ_m and Y_s at elevated temperature were not approximated by any equations.

Results and Discussion

The temperature effect on μ_m and Y_s is given in the usual representation in Fig. 1, and in Arrhenius form in Fig. 2. Linear parts of the plots in Fig. 2 were obtained by mathematical processing (see above). Corresponding exponential parts of the curves in Fig. 1 were plotted using Eqs. (3) and (4) and the values of constants E_μ, C_μ, E_Y, and C_Y obtained by the least-square technique. Ordinate scales in Fig. 1 are taken so that the heights of μ_m and Y_s maxima are similar (it is suitable for comparison of the curves).

Temperature dependences of μ_m and Y_s have much in common. Both plots are broken twice at similar temperatures: the first break at 30° C for μ_m and 28° C for Y_s, the second one at 36° C for μ_m and 35° C for Y_s. Taking into account that the fermentor

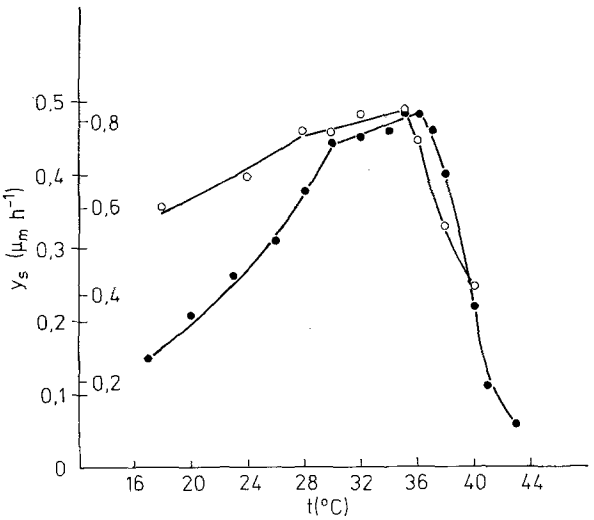

Fig. 1. Dependences of maximal specific growth rate, μ_m, and cell biomass yield, Y_s, of thermotolerant yeast *Candida valida* on temperature (conventional representation). μ_m, *closed circles left scale;* Y_s, *open circles right scale*

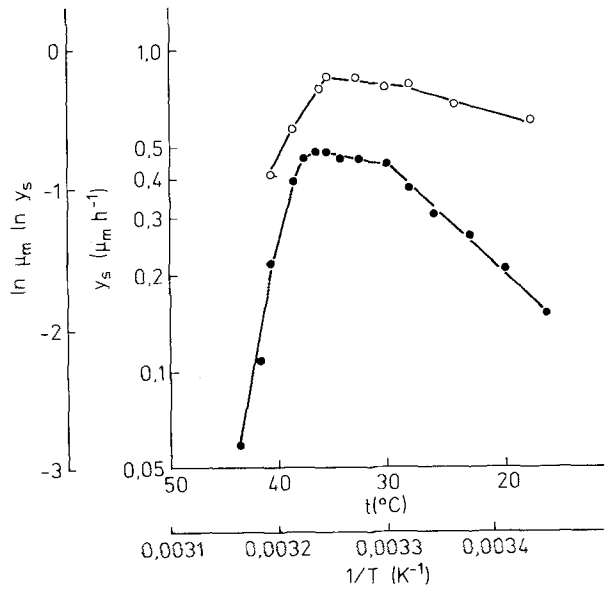

Fig. 2. Arrhenius plot for dependence of μ_m and Y_s of *Candida valida* on temperature. μ_m, *closed circles;* Y_s, *open circles*; ln, natural logarithm

temperature was maintained with the accuracy ± 0.5° C, one can consider differences between the corresponding break temperatures as insignificant. Each plot has three clearly distinguishable parts: (1) suboptimal region, where μ_m and Y_s fall with $t°$ decrease according to Arrhenius law; (2) optimal-temperature region, where Arrhenius law is still valid but both quantities are slightly dependent on $t°$; and (3) supraoptimal region, where μ_m and Y_s fall abruptly with $t°$ increase. The slope of both μ_m and Y_s is sharper at supraoptimal temperatures than at suboptimal ones.

[1] ln is natural logarithm

Table 1. Values of activation energies E_μ and E_Y for different ranges of the dependences of μ_m and Y_s on temperature

Micro-organism	Temperature range (° C)	E_μ (kJ · mol^{-1})	E_Y (kJ · mol^{-1})	References
Candida valida VKM-Y-2327	17–30	59.8	19.2	Our data
	30–36	8.16	7.84	
Bacillus caldotenax	37–45	196		Kuhn et al. (1980)
	45–60	75.7	8.3[a]	

[a] Activation energy for Y_s^m [see Discussion for Eq. (5)] measured at 50° C–60° C

Values of activation energy for μ_m and Y_s are presented in Table 1. For comparison the corresponding values (Kuhn et al. 1980) for the thermo philic bacterium *Bacillus caldotenax* are shown in this table. Comparison between such different organisms is interesting because it shows that the mechanisms underlying the mode of temperature effect on microbial growth are apparently similar.

The data obtained indicate two critical temperatures for *C. valida* growth, nearly 30° C and 36° C. At these temperatures the breaks on plots of μ_m and Y_s take place, one being caused by a sharp change of activation energy E, while the other by transition to the drop at supraoptimal temperatures. The detailed discussion of possible reasons of such transitions was presented by Kuhn et al. (1980). One of the reasons for E jump mentioned by Kuhn et al. is a change in metabolic regulations or in the physical structure of water occurring at 15° C, 30° C, 45° C, and 60° C. It should be noted that *C. valida* shows E jump at 30° C and *B. caldotenax* at 45° C, which is in line with the hypothesis of the influence of water structure transition. However, the values of E_μ at 30° C and 45° C differ markedly for both strains (see Table 1) and it is not clear whether it is possible to account for this difference on the basis of the structural porperties of water. Kuhn et al. (1980) showed that the break of μ_m and Y plots connected with transition to supraoptimal temperatures can be accounted for by a sharp increase in the death rate observed experimentally.

Figures 1 and 2 show that cell biomass yield is much less influenced by temperature than by the maximal specific growth rate. It is especially clear in the suboptimal and optimal regions, that is, at $t° < 36°$ C. The background of this difference could be connected with Y_s dependence on μ (Ierusalimsky 1963; Pirt 1965):

$$\frac{1}{Y_s} = \frac{1}{Y_s^m} + \frac{m_s}{\mu} \tag{5}$$

where m_s is the rate of substrate expenditure for cell maintenance, Y_s^m is the yield value in the absence of the mentioned expenditure, that is, the true growth yield.

Since in chemostat at each temperature we established the condition $\mu = \frac{1}{3}\mu_m$, it can be seen from Eq. (5), that during measurement of Y_s dependence on $t°$, the following interrelation took place:

$$\frac{1}{Y_s} = \frac{1}{Y_s^m} + 3\frac{m_s}{\mu_m} \tag{6}$$

The values of Y_s^m and m_s for the given strain were measured at 36° C during a separate chemostat experiment in which the yield was measured at different values of dilution rate D (Nguen et al. 1982). It was found: $Y_s^m = 0.81$, $m_s = 0.02\,\mathrm{h}^{-1}$. At 36° C $\mu_m = 0.48\,\mathrm{h}^{-1}$, from which: $1/Y_s^m = 1.23$, $3m_s/\mu_m = 0.12$. Thus the second term on the right side of Eq. (6) at 36° C is much smaller than the first one. Since both μ_m and m_s decrease as the temperature decreases (Kuhn et al. 1980), one can suppose that at temperatures lower than 36° C, both terms in Eq. (6) retain the same interrelation. If this is the case, the main part of the temperature effect on Y_s is based on Y_s^m dependence on $t°$, which is rather weak. The latter is probably connected with the fact that Y_s^m is a stoicheiometric quantity, unlike μ_m, which is a rate.

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