Effect of formaldehyde on kinetics of glucose consumption

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The effect of formaldehyde on kinetics of biomass growth and consumption of glucose by yeasts *Candida tropicalis* CCY 29-7-33 has been investigated. For modelling of kinetics of biomass growth and consumption of the substrate the models according to Monod, Contois, and Andrews were applied. The parameters of kinetic equations were determined using the combined explicit and implicit Euler method. To determine the minimum of the chosen function the simplex method of determination of the minimum of a nonlinear function with several variables without limitations was utilized. Of the models used to describe the kinetics of the process studied, the equation after Contois suited best. Comparable results were obtained also with the Monod's model. The results revealed the evident inhibitory effect of formaldehyde on growth of the biomass and consumption of the substrate. Increase of formaldehyde concentration was accompanied by decrease of specific growth rate values and increase of saturation constant values.

Исследовалось воздействие формальдегида на кинетику роста биомассы и потребления глюкозы дрожжами *Candida tropicalis* CCY 29-7-33. Для моделирования кинетики роста биомассы и потребления субстрата были применены модели, предложенные Моно, Контуа и Эндрюсом. Параметры кинетических уравнений были определены с использованием комбинированного эксплицитного и имплицитного метода Эйлера. Для определения минимума избранной функции был использован метод симплекса по определению минимума нелинейной функции с несколькими переменными без ограничений. Из всех моделей, примененых для описания кинетики изучаемого процесса, наиболее подходящим было уравнение Контуа. Сравнимые результаты были получены также с использованием модели Моно. Полученные результаты свидетельствуют об очевидном ингибирующем воздействии формальдегида на рост биомассы и потребление суб-

страта. Увеличение содержания формальдегида сопровождалось снижением величин специфической скорости роста и ростом значений константы насыщения.

Formaldehyde belongs to wide-spread products of chemical industry and, consequently, frequently occurs in waste waters. With regard to toxic and potentially mutagenic properties [1, 2], it represents one of the most spread compounds with undesired biological activity. The effect of formaldehyde on human health is discussed in detail in [3].

Formaldehyde is a membrane-active antimicrobial compound. It reacts with some functional groups of proteins and brings about their cross-linking [4]. Its main action is polycondensation, in consequence of which the structures become less permeable. Formaldehyde reacts also with nucleic acids and affects glycolysis [5].

On the other hand, formaldehyde is a physiological component of biological systems and plays an important role in methylation reactions of microorganisms, plants, and animals [6]. Formaldehyde is known as a central intermediate of the C_1 metabolism of methylotrophic yeasts [7—9]. In biological systems it arises from some endogenous and exogenous precursors. Formaldehyde formed endogenously is detoxicated in cells by incorporation into the C_1 pool *via* the tetrahydrofolate pathway and oxidized to formic acid and CO_2 [10]. Low nontoxic equilibrium concentrations of formaldehyde may be maintained in the cell.

Kinetics of biochemical degradation of formaldehyde has been the subject of several works, *e.g.* [11, 12]. The authors in [11] followed the kinetics of biodegradation of formaldehyde by the strain *Pseudomonas fluorescens* in the concentration range of 80—2000 mg dm^{-3}. To describe the kinetics of the process, they utilized the model including the effect of the substrate concentration, concentration of biomass, and concentration of the products. The authors in [12] studied the kinetics of biochemical decomposition of formaldehyde by activated sludge. From the results of their work it follows that the Vavilin's kinetic model is suitable to describe the kinetics of this process [13—15].

The aim of the present work is the study of the effect of formaldehyde on the growth of biomass and kinetics of consumption of glucose, the source of carbon and energy, by yeasts *Candida tropicalis* CCY 29-7-33, which are known to have broad assimilation spectrum.

Growth kinetics of microorganisms

Growth rate of microorganisms is described by specific growth rate, *i.e.* the mass of microorganisms formed per unit mass of microorganisms present. Its

value changes in the individual phases of growth curve. The specific growth rate value in the exponential phase is constant and can be expressed by the relation

$$\mu = \frac{1}{X}\frac{\mathrm{d}X}{\mathrm{d}t} \tag{1}$$

where μ is the specific growth rate [h^{-1}] and X is the concentration of microorganisms [$kg\,m^{-3}$].

The dependence of specific growth rate on concentration of the substrate is most often described by the Monod equation

$$\mu = \mu_{max}\frac{S}{K_S + S} \tag{2}$$

where μ_{max} is the maximum value of specific growth rate, S is the concentration of the substrate [$kg\,m^{-3}$], and K_S is the saturation constant [$kg\,m^{-3}$].

The Monod equation often adequately describes the kinetics of growth in a weakly inhibited medium. However, the specific growth rate may be influenced beside the substrate concentration also by concentration of the biomass, products, and inhibitors. In the literature there are reported series of kinetic models representing a modification of the Monod equation for conditions which are not considered in this equation [16—19]. For example, *Contois* explained the slowing of growth by "biological tightness" [16]. At high concentration of the biomass the cells interfere with each other and hinder their reproduction

$$\mu = \mu_{max}\frac{S}{KX + S} \tag{3}$$

where K is an empirical parameter. In many cases the main carbon and energy source at higher concentrations inhibits the growth and the specific rate can be expressed according to *Andrews* [17]

$$\mu = \mu_{max}\frac{S}{K_S + S + S^2/K_i} \tag{4}$$

where K_i is the constant of inhibition. Other kinetic equations for growth can be found in [18, 19].

Kinetics of substrate removal

The rate of concentration change of the substrate in dependence on the concentration change of the biomass can be expressed by the equation

$$\frac{\mathrm{d}S}{\mathrm{d}t} = -\frac{1}{Y}\frac{\mathrm{d}X}{\mathrm{d}t} \tag{5}$$

where Y is the coefficient of production of biomass [kg kg^{-1}]. Combination of eqns (*1*), (*2*), and (*5*) gives

$$-\frac{dS}{dt} = \frac{\mu_{max}}{Y}\frac{XS}{K_S + S} \tag{6}$$

By introducing the specific rate of substrate removal, eqn (*6*) can be rearranged

$$V_x = V_{x,max}\frac{S}{K_S + S} \tag{7}$$

where V_x is the specific rate of substrate removal [h^{-1}], $V_{x,max}$ is the maximum specific rate of substrate removal for which it holds

$$V_{x,max} = \frac{\mu_{max}}{Y} \tag{8}$$

Kinetic equations for substrate removal can be derived similarly from other kinetic models of growth, *e.g.* (*3*) and (*4*).

Experimental

Conditions of cultivation

To follow the effect of formaldehyde on kinetics of biomass growth and glucose consumption *Candida tropicalis* CCY 29-7-33 (Czechoslovak Collection of Yeasts, Institute of Chemistry, Slovak Academy of Sciences, Bratislava) was used. D-Glucose monohydrate, anal. grade, and 37 % aqueous solution of formaldehyde were used in the experiments. The yeasts were cultured in a synthetic medium of the following composition [20]: $(NH_4)_2SO_4$ (7.4 g), KH_2PO_4 (1.5 g), $MgSO_4 \cdot 7H_2O$ (0.5 g), $CaCl_2 \cdot 2H_2O$ (60 mg), $FeSO_4 \cdot (NH_4)_2SO_4 \cdot 6H_2O$ (12 mg), $ZnSO_4 \cdot 7H_2O$ (6 mg), $CuSO_4 \cdot 5H_2O$ (1 mg), and glucose (10 g) per 1 dm^3 of the medium, pH = 5.2, and vitamins added according to [21]. Cultivation of the inoculum in 500-cm^3 flasks that contained 100 cm^3 of the cultivation medium proceeded for 48 h at 28 °C with aeration on a rotary shaker (170 rev. min^{-1}). The effect of formaldehyde was followed by cultivation of *Candida tropicalis* (0.3 g dm^{-3} of the inoculum) in 500-cm^3 flasks that contained 100 cm^3 of the synthetic medium and formaldehyde (0—5 mmol dm^{-3}). In time intervals samples (2 cm^3) were withdrawn and A_{700} was measured on a Specol 10 apparatus in cell thickness 1 cm. The suspension of cells was centrifuged (4000 rev. min^{-1}) and in the supernatant glucose was determined with *o*-toluidine (BIO-LA test) and formaldehyde according to [22].

Determination of parameters of kinetic equations

The parameters μ_{max}, Y, and K_S of kinetic equations (*5*) and (*6*) were obtained by minimization of the function

$$\omega = \sum_{i=1}^{M} [(X_i^{exp} - X_i^{calc})^2 + (S_i^{exp} - S_i^{calc})^2] \qquad (9)$$

where M is the number of experimental measurements, X_i^{exp} and X_i^{calc} are respectively the measured and calculated concentrations of microorganisms of the i-th measurement, S_i^{exp} and S_i^{calc} are the measured and calculated substrate concentrations, respectively.

The values X_i^{calc} and S_i^{calc} were obtained by solution of differential equations (*5*) and (*6*). Eqn (*5*) was rearranged

$$X_{t+\Delta t} = X_t - Y(S_{t+\Delta t} - S_t) \qquad (10)$$

Eqn (*6*) was solved by the combined explicit and implicit Euler method [23]

$$S_{t+\Delta t} = S_t - 0.5 \frac{\mu_{max}}{Y} \frac{X_t S_t}{K_S + S_t} \Delta t - 0.5 \frac{\mu_{max}}{Y} \frac{X_{t+\Delta t} S_{t+\Delta t}}{K_S + S_{t+\Delta t}} \qquad (11)$$

With regard to $S_{t+\Delta t}$ eqn (*11*) is nonlinear. For the solution of this equation we used the Newton's method [19]

$$S_{t+\Delta t,k+1} = S_{t+\Delta t,k} - \frac{f_1(S_{t+\Delta t,k}, X_{t+\Delta t,k})}{f_1'(S_{t+\Delta t,k}, X_{t+\Delta t,k})} \qquad (12)$$

where $S_{t+\Delta t,k+1}$ denotes the substrate concentration at time $t + \Delta t$ in the $(k+1)$-th iteration of the solution of eqn (*11*), $S_{t+\Delta t,k}$ is the analogue of $S_{t+\Delta t,k+1}$ for the k-th iteration, $f_1(\ldots)$ and $f_1'(\ldots)$ denote respectively the function and the derivation of the function, which is obtained by rearrangement of eqn (*11*) so that the expression on the right-hand side is to be equal to zero. The first estimation for the solution after eqn (*12*) was determined by the solution of eqn (*6*) using the Euler's explicit scheme [23]. Iteration calculation after eqn (*12*) was completed in the case when

$$|S_{t+\Delta t,k+1} - S_{t+\Delta t,k}| \leq \varepsilon$$

where ε is the required accuracy of the solution. The time change Δt was chosen so that calculation be by an order more accurate than the accuracy of measurements. The accuracy of calculation was checked by comparison of the results of calculation with those of the twofold higher step. The calculated values of concentrations both of the substrate and microorganisms were tabulated in time

instants t_i, equal to time intervals whithin which the experimental measurements were carried out. The minimum of the function ω, given by relationship (*9*), was determined by the simplex method for determination of the minimum of a nonlinear function with several variables without limitations [24]. We proceeded similarly also in determining the parameters of kinetic equations derived from the relationships for specific rate according to Contois (eqn (*3*)) and Andrews (eqn (*4*)).

Results and discussion

To generalize the description of kinetics of growth of yeasts *Candida tropicalis* CCY 29-7-33 and glucose consumption at conditions when glucose was the source of carbon and energy and formaldehyde (c = 0—5 mmol dm^{-3}) was present in the growth medium, three kinetic models, the relationships according to Monod, Contois, and Andrews (eqn (*2*—*4*)), were used. It was presumed that they might adequately describe the growth kinetics in a weakly inhibited medium. From Fig. 1 it is obvious that the presence of formaldehyde brings about a prolongation of lag-phase, however, in the presence of a metabolically

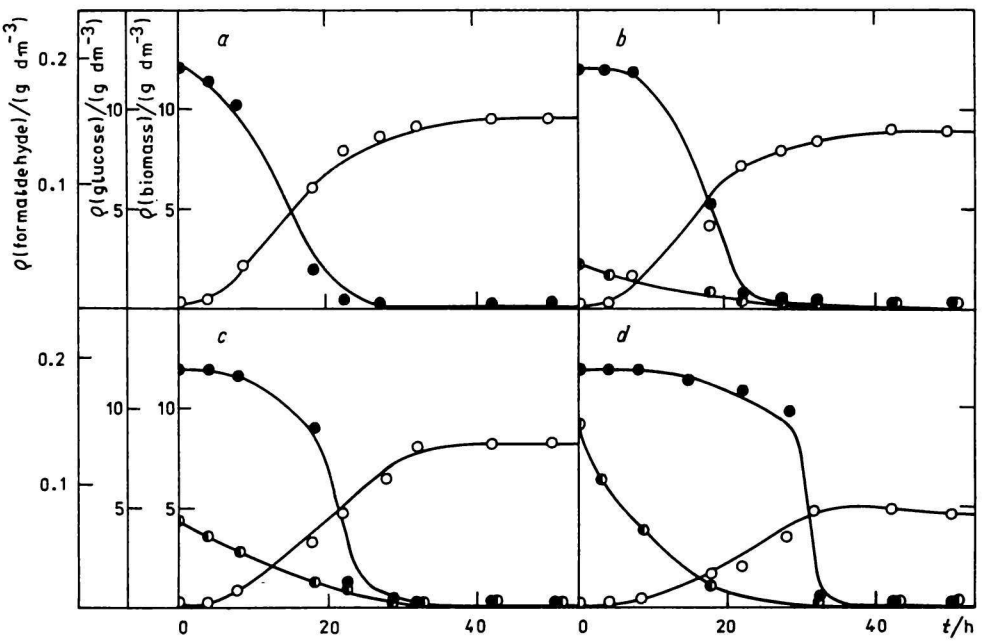

Fig. 1. Effect of formaldehyde on growth of *Candida tropicalis* CCY 29-7-33 in a synthetic medium with glucose (ϱ = 12 g dm^{-3}) (*a*) and addition of formaldehyde: c = 1.25 mmol dm^{-3} (*b*); c = 2.5 mmol dm^{-3} (*c*), c = 5.0 mmol dm^{-3} (*d*); pH = 5.2 under aeration at 28 °C.
○ Increase of biomass, ● decrease of glucose, ◐ decrease of formaldehyde.

Table 1

Values of biokinetic parameters and function (*9*) (c(formaldehyde) = 0.0 mol dm^{-3})

Kinetic model	μ_{max} / h^{-1}	Y / kg kg^{-1}	K_S / kg m^{-3}	K	K_i / kg m^{-3}	Function (*9*)
Monod	0.2812	0.6996	4.6644	—	—	3.1232
Contois	0.2296	0.7062	—	0.8101	—	2.7592
Andrews	0.2002	0.6695	5.2233	—	1.3650	12.4100

active yeasts-culture, it is biologically degraded and within 48 h its concentration decreases practically to zero. The values of parameters of the chosen kinetic relationships and the values of function (*9*) for experimental measurements in the absence of formaldehyde are presented in Table 1. The course of experimental and calculated values of the substrate and biomass concentrations is illustrated in Fig. 1*a*. It is evident from Fig. 1*a* and from the values of the function, which shows the closeness of experimental and calculated values (Table 1), that the experimental measurements in the case of glucose without formaldehyde are best described by the relationship according to Contois (eqn (*3*)). Comparable

Table 2

Values of biokinetic parameters and function (*9*) in dependence on concentration of formaldehyde (Monod's model — eqn (*2*))

Concentration of formaldehyde / mol dm^{-3}	μ_{max} / h^{-1}	Y / kg kg^{-1}	K_S / kg m^{-3}	Function (*9*)
0.00125	0.2454	0.6774	4.7863	4.6947
0.0025	0.2372	0.6416	6.5502	18.8647
0.0050	0.1568	0.4810	7.8067	45.8210

Table 3

Values of biokinetic parameters and function (*9*) in dependence on concentration of formaldehyde (Contois' model — eqn (*3*))

Concentration of formaldehyde / mol dm^{-3}	μ_{max} / h^{-1}	Y / kg kg^{-1}	K	Function (*9*)
0.00125	0.1607	0.6708	0.7775	3.4435
0.0025	0.1570	0.6285	0.6165	19.2081
0.0050	0.0881	0.4680	0.1153	38.9689

results were obtained also when using the Monod's relationship. The values of biokinetic parameters and of the function (*9*) obtained by treatment of kinetic measurements at different concentrations of formaldehyde are presented in Tables 2 and 3. The course of the values calculated according to the Contois' relationship and the experimental values for glucose in the presence of formaldehyde ($c = 1.25$ mmol dm^{-3}) is illustrated in Fig. 2*b*. From the results presen-

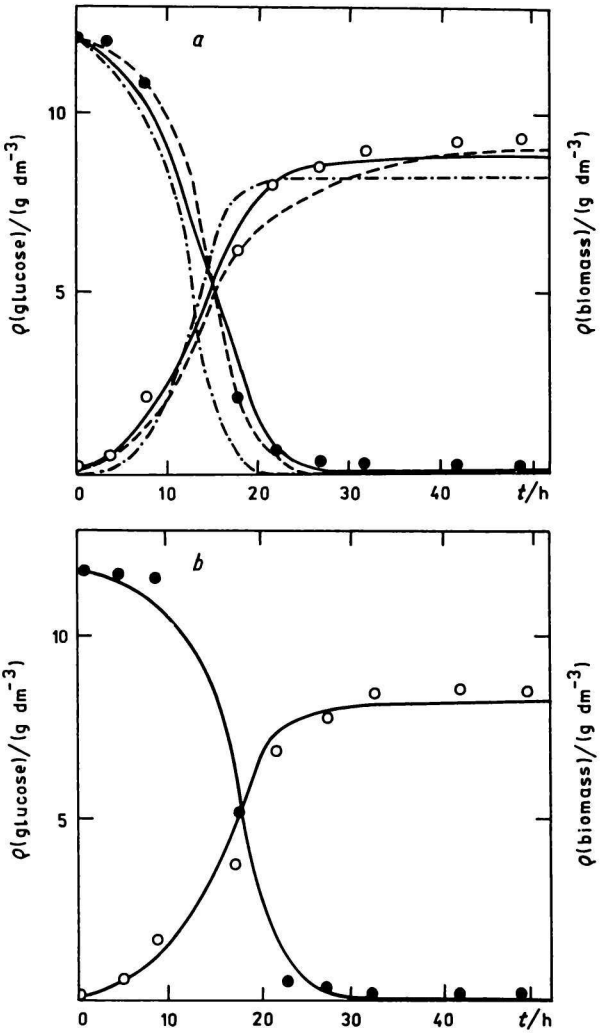

Fig. 2. Course of the measured and calculated values of biomass growth and substrate consumption in cultivation of *Candida tropicalis* CCY 29-7-33 in a synthetic medium with glucose ($\varrho = 12$ g dm^{-3}) in the absence (*a*) and in the presence (*b*) of formaldehyde ($c = 1.25$ mmol dm^{-3}). ● S^{exp}, ○ X^{exp}, – – – Monod's model, ——— Contois' model, – · – · – Andrews' model.

ted in Tables 2 and 3 it follows that with the increasing concentration of formaldehyde the values of specific growth rates of the biomass decrease. Analogously can be observed a decrease of the values of the coefficient of biomass production and an increase of the saturation constant values for the Contois and Monod's models. A similar trend was observed also in the case of using the relationship according to Andrews (eqn (*4*)), while the values of the function (*9*) were by an order higher.

Conclusion

The results of treatment of kinetic experimental measurements pointed to inhibitory effect of formaldehyde on the growth of *Candida tropicalis* CCY 29-7-33 and glucose consumption in the presence of formaldehyde ($c = 0$—5 mmol dm^{-3}). It was reflected in decrease of the specific growth rate values, decrease of the values of the coefficient of biomass production, and increase of the saturation constant values with the increasing concentration of formaldehyde. For mathematical description of the kinetics of biomass production and substrate consumption by the yeasts-culture studied, the Contois' relationship suited best. Comparable results were obtained also in the case when using the Monod's relationship.

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