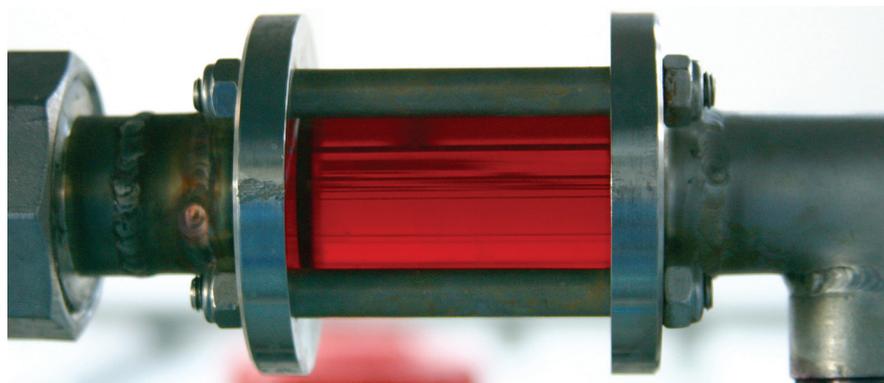


A New Kinetic Structured Model for Cell Cultivation in a Chemostat

Sergey P. Klykov and Vladimir V. Kurakov

Mathematically modeling the kinetics of batch and continuous cultivation allows you to not only calculate and evaluate the effects of process parameters, but also to forecast those parameters and duration of cultivation to develop a cost-effective production process. Mathematical modeling microorganism cultivation was intensively developed in the second half of the last century.

Monod proposed Equation 1 for batch processes in 1942 (1). In that equation, μ and μ_{\max} are the specific and maximum growth rates, respectively (h^{-1}), S is the substrate concentration, (g/L), and K_s denotes a Monod semisaturation constant (g/L). That model implies that yield Y is a constant value. For continuous-mode cultivation (e.g., chemostat), Y can be calculated using Equation 2, in which X is the biomass concentration (g/L), and S_0 is the limiting substrate concentration in the input (g/L). For a continuous cultivation at flow rate D



(WWW.SXC.HU)

(h^{-1}), combined solution of Equations 1 and 2 gives the equation called a *Monod model* for chemostat cultivation under full stirred conditions and substrate limitation. For the biomass, this is Equation 3, and for the limiting substrate it is Equation 4.

In 1959, Luedeking and Piret (2) presented a model describing cell cultivation to produce valuable metabolites. Moser (3) in 1958 and Andrews (4) in 1968 both described models for continuous processes, taking into account substrate inhibition as an example of trying to resolve some limitations of the Monod theory. Their work is shown as Equations 5 and 6, in which K^* denotes a new parameter not considered in Equation 1, provided that $K^* > 1$, and K_i is the inhibition constant.

The improved Monod equation and kinetic models on its base describe biomass growth and formation of target products gave rise to various stochastic, unstructured, and

Equations 1–6

Equation 1

$$\mu = \frac{\mu_{\max} S}{K_s + S}$$

Equation 2

$$X = Y(S_0 - S)$$

Equation 3

$$X = \frac{Y(S_0 - DK_s)}{(\mu_{\max} - D)}$$

Equation 4

$$S = \frac{DK_s}{(\mu_{\max} - D)}$$

Equation 5

$$\mu = \frac{\mu_{\max} S^{K^*}}{K_s + S^{K^*}}$$

Equation 6

$$\mu = \frac{\mu_{\max} S}{K_s + S + \frac{S^2}{K_i}}$$

structured models, which Gormely reviewed in detail in 1973 (5). Pirt (6), Bailey and Ollis (7), and Terskov and Gurevich (12) presented the classification and critical analysis of the developed kinetic models. Some studies have linked process kinetics to the physiological state of cells and to the concentration of substrates consumed for biomass growth and maintenance of population viability (8–11, 13–24).

PRODUCT FOCUS: RECOMBINANT PROTEINS

PROCESS FOCUS: PRODUCTION

WHO SHOULD READ: PROCESS DEVELOPMENT AND CHARACTERIZATION

KEYWORDS: KINETICS, PERFUSION, SIMULATION, MODELING, FERMENTATION, OPTIMIZATION

LEVEL: ADVANCED

Table 1: Model parameters and significant factors for the calculation of various types of static characteristics

Shape of Static Characteristics Curves	$X_{D_{max}}$ (g/L)	$X_{(D \rightarrow 0)}$ (g/L)	$D_{max} = Yk^{div} \approx \mu_{max}^*$ (h ⁻¹)	Yk^{st} (h ⁻¹)	Sign in Equations 20, 21
Conformity with the Monod model	0.65	1.5	1.111	0.518	+
Deviation 1 from the Monod model 1) For $D > \mu_{max}$ $X > 0$ 2) For $D \leq \mu_{max}$ A limiting factor of cell growth enters the system regardless of medium flow.	0.28	0.65	1.111	0.759	+
Deviation 2 from the Monod model; Substrate inhibition and endogenous metabolism	0.65	1.5	1.111	0.518	-
Deviation 3 from the Monod model; Presence of the inhibitor in the nutrient medium	0.28	0.65	1.111	0.363	+
Deviation 4 from the Monod model; Inhibition by the product	3	6	0	0.518	-
Deviation 5 from the Monod model; Oxygen limitation	5	10	0	0.518	-
Deviation from the Monod model; Endogenous metabolism	0.65	1.5	0	0.518	+

Here we discuss the method of structuring a cell population into dividing and nondividing cells of zero age. We describe universal laws deduced mathematically and show graphically the types of static characteristics of continuous-mode cultivation $X = f(D)$. The Monod equation and the other well-known models are the particular cases of our proposed model. In addition, we provide some discrepancies of the proposed method from the Monod model:

- Extreme dependence of a specific growth rate on substrate concentration implies a maximum specific growth rate, which differs from the results obtained from the Monod equation.
- Specific growth rate depends on inhibitory metabolite concentration, which differs from the proposed model.
- The proposed model describes energy (oxygen) limitation when the concentration of substrate (oxygen) is very low due to its extremely low solubility. The Monod model and others do not consider such a case.

MATERIALS AND METHODS

Our previous structured approach for calculating the model and to control batch processes is based on two differential equations for biomass growth inhibition phase (GIP) (13–16). For the biomass, Equation 7 applies. In that equation, n is the integer, an order of the derivative of the function;

Equations 7–14:

Equation 7

$$\frac{d^n X^{div}}{d(X^{st})^n} = \frac{K}{A^2} \frac{(-1)^{(n-1)} n!}{(X^{st})^{(n+1)}} - C$$

Equation 8

$$d[P \text{ or } (-S)]/dt = k_{p_s}^{div} X^{div} + K_{p_s}^{st} X^{st}$$

Equation 9

$$q = k_p^{div} + (k_p^{st} - k_p^{div})R$$

Equation 10

$$q = (-dS/dt) * (1/X)$$

Equation 11

$$Y = -dX/dS$$

Equation 12

$$D = \mu$$

Equation 13

$$qY = (-dS/dt) * (1/X) * (-dX/dS) = Yk^{div} + Y(k^{st} - k^{div}) * R = \mu = D$$

Equation 14

$$\mu_{max} \rightarrow Yk^{div} = D_{max}$$

X^{div} is the quantity of dividing cells; X^{st} is the quantity of nondividing (stable) cells; K is a product of overall growth rate and the rate of accumulation of stable or nondividing cells; A is the ratio of maintenance energy to the energy consumed for biomass growth or a specific rate of accumulation of stable or nondividing cells. Furthermore, $C = 1$ if $n = 1$, and $C = 0$ if $n \geq 2$.

For the target product (substrate), Equation 8 applies. In that equation, P is the target product, S denotes the limiting substrate, $k_{p_s}^{div}$ (h⁻¹) and $k_{p_s}^{st}$ (h⁻¹) are the product and

substrate constants of dividing and nondividing cells, respectively.

To get a generalized model of the chemostat, we will use equations for the total amount of biomass in GIP, a share of nondividing cells ($R = X^{st}/X$, in which X is the total biomass for GIP), changes in the number of nondividing cells and their share $X^{st}R$ at the end of logarithmic growth phase (LGP), and a specific rate of product formation and substrate consumption (Equation 8). This is Equation 9, in which q is defined by Equation 10.

Yield is determined by Equation 11. In a steady state, flow rate is equal to the specific growth rate (Equation 12) (1–6).

Under equilibrium conditions, Equations 9 and 10 may be equated. Then multiplying the left and right sides of the equation for Y (Equation 11), we obtain the final equation for the dependence of flow rate on the number of nondividing cells and yield (Equation 13). As we show below, Equation 13 represents a generalized equation. It combines all the cases of culture behavior in all flow processes, and describes all deviations from the Monod model.

Clearly, there are no limits for exponential cell growth. The rate of substrate consumption is maximum and is expressed by k^{div} , provided that maximum specific growth rate μ_{max} follows Equation 14. In such case, it is

Equations 15–23:

Equation 15 Equation 16

$$R = 2X_1(1/X - X_1/X^2) \quad X_1 = X_{Lim}/X_p$$

Equation 17

$$R = \frac{K}{A^2(X_p * X - X^2)}$$

Equation 18

$$D - Yk^{div} = 2Y(k^{st} - k^{div})X_{Dmax} \frac{1}{X} - 2Y(k^{st} - k^{div})X_{Dmax}^2 \frac{1}{X^2}$$

Equation 19

$$D - Yk^{div} = \frac{Y(k^{st} - k^{div})K}{A^2(X_{(D \rightarrow 0)}X - X^2)}$$

Equation 20

$$(D - Yk^{div})X = 2Y(k^{st} - k^{div})X_{Dmax} \frac{1}{X} - 2Y(k^{st} - k^{div})X_{Dmax}^2 \frac{1}{X^2}$$

Equation 21

$$\frac{1}{(D - Yk^{div})X} = \frac{A^2X_{(D \rightarrow 0)}}{KY(k^{st} - k^{div})} - \frac{A^2X}{KY(k^{st} - k^{div})}$$

Equation 22

$$X = \left[\frac{1}{2X_{Dmax}} + \sqrt{\frac{1}{4X_{Dmax}^2} - \frac{(D - Yk^{div})}{2Y(k^{st} - k^{div})X_{Dmax}^2}} \right]^{-1}$$

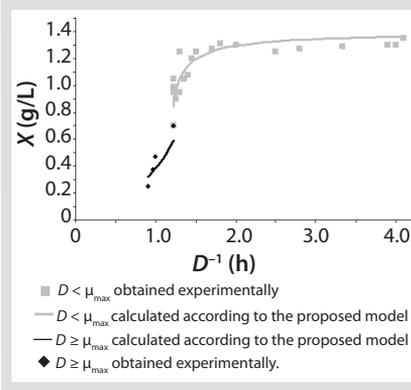
Equation 23

$$X_{1,2} = \frac{X_{(D \rightarrow 0)}}{2} \pm \sqrt{\frac{X_{(D \rightarrow 0)}^2}{4} - \frac{Y(k^{st} - k^{div})K}{A^2(D - Yk^{div})}}$$

impossible to control the chemostat, and biomass concentration in the stationary growth phase may take any arbitrary value (7).

If growth rate is limited by some substrate, then nondividing cells must appear in the system. Such cells possess a limiting substrate consumption distinctive rate k^{st} that affects the static characteristics. Previous studies showed that nondividing cells can be formed at the end of LGP during batch fermentation (13–16). We show below that this very phenomenon accounts for the observed deviations from the Monod model and is expressed in the absence output of microorganisms predicted by the Monod model when the rate of dilution D is equal to or significantly higher than μ_{max} . So, we must examine cultivation at $D \geq \mu_{max}$ and $D < \mu_{max}$. In previous studies (13–16), we used Equations 15–17 for R at the end of LGP and GIP. In those equations, X denotes the total biomass concentration in the batch process, and X_1 (defined by Equation 16) is the

Figure 1: Data on *Aerobacter cloacae* growth in a batch culture.



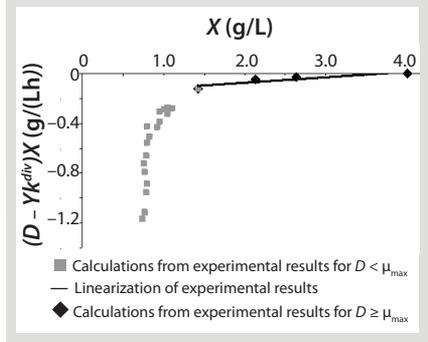
biomass concentration at the beginning of population structuring.

Equations 15 and 17 correspond to $D \geq \mu_{max}$ and $D < \mu_{max}$, respectively. Parameters $X_{(D \rightarrow 0)}$ and X_{Dmax} correspond to parameters X_p and X_{Lim} , respectively during batch fermentation in the chemostat. The values of $X_{(D \rightarrow 0)}$ are always greater than those of X_{Dmax} for all types of static characteristics (such as $D < \mu_{max}$), except for “endogenous metabolism” characteristics (9) for which $X_{(D \rightarrow 0)} < X_{Dmax}$. Substitution of Equations 15 and 17 into Equations 13 and 15 shows the dependence of flow rate D on the equilibrium concentration of the biomass X for cases in which $D \geq \mu_{max}$ and $D < \mu_{max}$. The results are Equations 18 and 19.

If the left and right sides of Equation 18 are multiplied by X , and Equation 19 is converted to $((D - Yk^{div}) * X)^{-1} = f(X)$, and both sides of the resulting expression are divided by X , then the resulting equations (20 and 21) of the structured model determine static characteristics of the chemostat.

If dependencies of the experimental data are built according to Equations 20 and 21 in the coordinates $(D - Yk^{div}) * X = f(X^{-1})$ and $((D - Yk^{div}) * X)^{-1} = f(X)$, then it would be possible to work out all the parameters of the chemostat structured model. Parameters μ_{max} , Y , S_0 , X_{Lim} , X_p , A , and K/A^2 can be determined first in the batch process. The points of those graphical dependencies, which are described by relevant laws, should be well grouped around a straight line. Deviations from the straight line

Figure 2: Parameters of static characteristics of $D \geq \mu_{max}$; line is $(D - Yk^{div})X = 0.0547X^{-1} - 0.199$.



indicate the action of one of the above lows. Common chemostat models represent static processes as a function $X = f(D)$. So, Equations 20 and 21 have been converted in accordance with a generally accepted view. For $D \geq \mu_{max}$ and $D < \mu_{max}$, these equations appear, respectively, as Equations 22 and 23. In Equation 23, X_1 has a positive (+) solution to match the Monod model, and X_2 has a negative (-) solution for cultures with “endogenous metabolism” in compliance with results from Bailey and Ollis (7).

Equation 19 can be used to calculate a low boundary of the chemostat operating range (the admissible value D at which the equilibrium concentration of the biomass will be guaranteed to match the values of X being set by the proposed chemostat model). For the static characteristics of the type $D \leq \mu_{max}$, this value corresponds to $D = Yk^{st}$. Using Equation 23, you can determine the equilibrium concentration of biomass X for the upper boundary of the permissible flow rate in the chemostat, when $D \leq \mu_{max}$, at $D = \mu_{max}$. The former value is optimal in terms of biomass as a target product because it ensures maximum productivity. In practice, it often happens that when $D = \mu_{max}$, equilibrium biomass concentration $X = 0.5X_{(D \rightarrow 0)}$, lower as it was shown in the example of “Conformity with the Monod model” (Table 1). As a rule, any deviation from the boundaries of the determined range can lead to uncontrollable changes in the equilibrium states. However, remember that the state $D = \mu_{max}$ is

impossible in all processes. To control such processes, the parameter concentration S_0 in the input flow, should be taken into account. Also, the quality of the nutrient medium composition should cause concern because possible growth inhibitors may be present in it.

RESULTS AND DISCUSSION

Equation 13 as a Generalized Model with respect to Earlier Models:

In the proposed model described by Equations 13, 22, and 23, the main determining factors in the system under equilibrium conditions are constants of substrate use by appropriate cell groups as well as the relative number of nondividing cells R both in the steady state and in the initial batch culture. This model allows for better understanding and control of the essential elements in continuous processes. The essence of the chemostat state is that the derivative of the dividing cells with respect to nondividing cells is a fixed constant for any a specific value D . That is a more reasonable and precise condition than that of Equation 12, because if $D > \mu_{max}$ (as it was deduced in generally accepted models such as the Monod model), then culture process management is impossible, and the situation itself is by no means explained. Table 1 lists examples of possible static characteristics of continuous cultures that describe and explain the structured chemostat models (Equations 22 and 23) at a qualitatively new level. It is important that the Monod model can be derived from the general model (Equation 13, assuming that $D \leq \mu_{max}$), provided that the equation is reduced to a common denominator and

- R is substituted from Equation 17
- X from Equation 4 replaces S
- it is assumed that $S \ll S_0$.

That simplification leads to Equation 24. In that equation, taking into account $S \ll S_0$ in the denominator, the values $X_{(D \rightarrow 0)} - YS_0$ and YS are comparable. So, the value S in the expression in brackets in the denominator and numerator can not be equated to 0. The first two terms

in the numerator represent the sum of the constants, and if its sum is much less than the third term in the numerator containing multiplier S , then it can be equated to 0. In this case, for the first two terms in the numerator Equation 25 is true.

Reduction of both the numerator and denominator in Equation 24 by $S_0 Y^2$, replacement of D for μ , and use of Equation 12 leads to Equation 26. That equation is fully consistent with the Monod Equation 1, in which the constant K_s is defined by equation 27.

Similar to the deductions of the Monod model, using Equations 12 and 14 to make the appropriate substitutions in Equations 20 and 21 and simplifying the deduced ratios results in a model of the dependence of specific growth rate on the substrate

Equations 24–31:

Equation 24

$$D = \frac{[Y^2 A^2 k^{div} (X_{(D \rightarrow 0)} - YS_0) S_0 + Y(k^{st} - k^{div}) K + Y^3 A^2 k^{div} S S_0]}{A^2 (X_{(D \rightarrow 0)} - YS_0 + YS) Y S_0}$$

Equation 25

$$Y^2 A^2 k^{div} (X_{(D \rightarrow 0)} - YS_0) S_0 + Y(k^{st} - k^{div}) K = \text{Constant}$$

$$\text{Constant} \ll Y^3 A^2 k^{div} S S_0 \Rightarrow$$

$$Y^2 A^2 k^{div} (X_{(D \rightarrow 0)} - YS_0) S_0 + Y(k^{st} - k^{div}) K \rightarrow 0$$

Equation 26

$$\mu = \frac{Y k^{div} S}{\frac{X_{(D \rightarrow 0)} - YS_0}{Y} + S} = \frac{\mu_{max} S}{K_s + S}$$

Equation 27

$$K_s = \frac{X_{(D \rightarrow 0)} - YS_0}{Y}$$

Equation 28

$$\mu = \mu_0 + \mu_1 \frac{S}{K_s + S}$$

Equation 29

$$K_s = S_0 \frac{YS_0 + X_{(D \rightarrow 0)}}{2YS_0 - X_{(D \rightarrow 0)}}$$

Equation 30

$$\mu_0 = Y k^{div} \frac{X_{(D \rightarrow 0)}}{2YS_0 - X_{(D \rightarrow 0)}}$$

Equation 31

$$\mu_1 = Y k^{div} \frac{YS_0 - X_{(D \rightarrow 0)}}{YS_0 - \frac{1}{2} X_{(D \rightarrow 0)}}$$

concentration proposed by Pirt (6), shown as Equation 28. In that equation, μ_0 is the specific growth rate at zero concentration of the substrate,

Figure 3: Parameters of static characteristics at $D < \mu_{max}$; line is $((D - Yk^{div})X)^{-1} = 6.2335X - 9.3302^*$

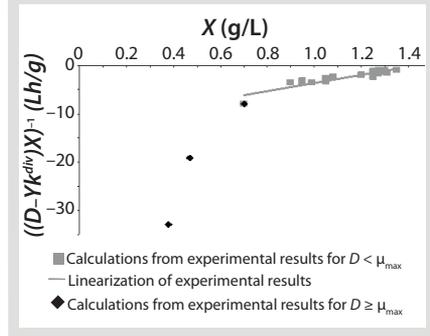
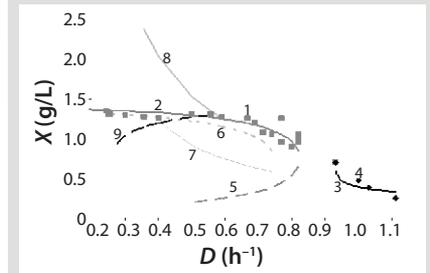
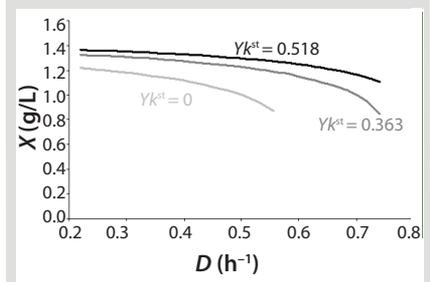


Figure 4: Description of the proposed model for all possible experimental stationary biomass concentrations depending on the flow rate D^*



* 1 = calculations from experimental results for $D < 0.88 \text{ (h}^{-1}\text{)}$; 2 = Compliance with the Monod model, model calculations for $D < 0.88 \text{ (h}^{-1}\text{)}$, $k^{div} > k^{st}$; 3 = deviation 1 from the Monod model, synchronous input culture, model calculations for $D \geq 0.88 \mu\text{ac}^{-1}$, $k^{div} > k^{st}$; 4 = deviation 1 from the Monod model, experimental results for $D \geq 0.88 \text{ (h}^{-1}\text{)}$; 5 = deviation 2 from the Monod model, substrate inhibition, endogenous metabolism according to Reference 7, $D < 0.88 \mu\text{ac}^{-1}$, $k^{div} > k^{st}$; 6 = deviation 3 from the Monod model, processes in the presence of the inhibitor in the medium. $k^{div} \gg k^{st}$; 7 = deviation 4 from the Monod Model calculations for $D < 0.88 \mu\text{ac}^{-1}$, $k^{div} = 0$, inhibition by the product; 8 = deviation 5 from the Monod model, model calculations for $D < 0.88 \mu\text{ac}^{-1}$, $k^{div} = 0$, $X_{p(8)} \gg X_{p(2)}$, oxygen limitation; 9 = Deviation 6 from the Monod model, model of slow growth, $k^{div} = 0$, Endogenous metabolism according to Reference 12.

Figure 5: Decrease of static characteristics for various k^{st} at constant k^{div}



and the specific rate ($\mu_0 + \mu_1$) is reached when $S \rightarrow \infty$. If Equation 17 is substituted into Equation 21, then by rearranging terms and reducing the known small values that contain the parameter S^2 and the constant K , we form an equation that is formally identical to the Pirt model for specific growth rate. Using the obtained equation K_s , μ_0 , and μ_1 can be defined as Equations 29–31. Those equations — which are similar to those of Moser (Equation 5) and Andrews (Equation 6) — can be deduced from Equation 13 for the structured model. An equation for the processes of product inhibition — such as the Ierusalimsky model (12) — can be similarly deduced.

When applying this method, you should remember that it describes static characteristics, which are essentially a set of various processes running at different rates D . For continuous-mode cultivation, it is correct to say that $D \rightarrow 0$ rather than $D = 0$, because a simple substitution of $D = 0$ in the equation for the “flow” model does not mean that equations for batch processes can be automatically obtained in this way. Thus, the proposed continuous-mode cultivation model is based on the subdivision of the microbial population into dividing and nondividing cells of zero age. The model takes into account different rate constants for the consumption of a limiting substrate and constants for a biomass. It also explains the well-known phenomenon of chemostat behavior deviation, which has not been described precisely by mathematical methods (12) that make cultivation control of microbiological processes impossible. Our proposed mathematical model of the chemostat will help to change the situation.

Examples of Calculations of Various Static Characteristics from Equations 21–23: Table 1 lists the model parameters and significant factors for the calculation of various types of static characteristics. Figure 1 shows the literature data (7) for calculating a continuous process for *Aerobacter cloacae* and the corresponding curves calculated from the shown models. Table 1 shows the the data as “Conformity with the Monod model” and “Deviation 1 from the Monod

model.” We calculated the parameters of those models after constructing dependences shown in Figures 2 and 3, specified in Equations (21 and 23) and (20 and 22), respectively for each case.

Table 1 shows the parameters for other possible types of static characteristics of continuous cultures analyzed in detail in a previous study (12). Table 1 and the legend of Figure 4 (numbers 2–6) show the deviations from the Monod model. We arbitrarily set Model parameters for such deviations (except for deviation 2, for which we used the parameters of the curve for “Conformity with the Monod model” and a “–” sign for the solution). The only condition was that the shape of the derived characteristics would strictly conform to the shape of the curve presented in reference 12. Table 1 also shows the significant factors (“+” or “–” sign) that we used to solve Equation 23 and construct Figure 4.

Figure 5 shows the decrease of the static characteristics for different k^{st} values at constant k^{div} for $D \leq \mu_{max}$, as frequently encountered in microbiological practice. In all those examples, $Y = \text{constant}$. The calculation of the relevant characteristics of the substrate is not difficult. For all subsequent examples, the constant yield is used on the default. Therefore, experimental results comply with model data.

OUR PROPOSED MODEL

We propose a new mathematical model for cell cultivation in a chemostat. Our model is based on the structuring of the biomass into two main groups: dividing and nondividing cells. The model is applicable both to existing static characteristics such as the Monod model and to the deviations from it.

We determined the range of chemostat stability at the specified flow rate D and concentration of the input limiting substrate S_0 . We also proposed the methods for determining parameters of the chemostat structured model.

The value of the derivative of dividing cells to nondividing cells of zero age is constant for a given flow rate. That value is no less important

than the equality of specific growth rate and nutrient flow rate, which determines the equilibrium of the chemostat.

We showed that the corresponding specific rate constants of limiting substrate use by dividing and nondividing cells determine the system equilibrium. We also demonstrated that the new proposed structured model of the chemostat is more general than any other existing model. In each specific case, the model provides comparable equations to the well-known models of Monod, Pirt, Moser, Andrews, and Ierusalimsky.

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