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original scientific paper

Mathematical Modelling of Antibiotic Biosynthesis Kinetics and its Relevance to the Process Design and Optimization*

Matematičko modeliranje kinetike biosinteze antibiotika i njegovo značenje za projektiranje i optimiranje procesa

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Summary

The use of mathematical models in describing kinetics of antibiotic production processes is discussed. The special emphasis is given to models with respect to their role in facilitating the understanding, design, optimization and control of microbial processes. The processes of oxytetracycline and erythromycin biosynthesis where analysed applying our own already developed mathematical model. Since the model was found to fit well to experimental data it was applied to simulate the process course of supposed different cultivation conditions. The applied model was found to be convenient for use in finding the way of improved oxytetracycline production and in improving the method of microorganism selection.

Sažetak

Raspravlja se primjena matematičkih modela pri opisu kinetike procesa proizvodnje antibiotika. Posebna važnost dana je modelima s obzirom na njihovu ulogu u olakšavanju spoznavanja, projektiranja, optimiranja i vođenja mikrobnih procesa. Procesi biosinteze oksitetraciklina i eritromicina analizirani su primjenom već razvijenog vlastitog matematičkog modela. Kako je ustanovljena dobra usklađenost modela s eksperimentalnim podacima, on je upotrijebljen za kompjutorsku simulaciju tijeka procesa uz pretpostavljene različite uvjete uzgoja. Ustanovljeno je da je primijenjeni model prikladan za pronalaženje djelotvornijeg načina proizvodnje oksitetraciklina i za poboljšanje odabira mikroorganizama.

Introduction

Design and optimization of microbial processes depend very largely on the information representing all our knowledge on mechanism and kinetics of particular microbial processes. Complexity is common property of all microbial processes. However, in contrast to the relatively simple production of those metabolites which are synthesized during the trophophase in connection with the biomass reproduction process, i. e. in the case of production of »primary metabolites«, more complex is the production of such metabolites the formation of which is not directly linked to the growth of the culture. These metabolites are defined as »secondary metabolites« and for them it can be considered »everything that is not used by the cell as an inevitable constituent of the reproduction cycle« *(1)*. The great majority of discovered antibiotics is produced by fermentation processes, where they appear as secondary metabolites of different microbial species.

Mathematical models appear as an useful tool in describing kinetics of microbial processes and facilitating the understanding, design, optimization and control of microbial processes, disregarding the fact that any mathematical model represents only the overall or macroscopic behaviour of the system. There is the information on a number of attempts of applying mathematical models to processes of antibiotic synthesis. It could be of importance to give a brief piece of information on some of them here. Aiba and Hara *(2,3)* proposed the concept of mean cumulative age in order to explain biomass physiology and productivity of batch and continuous cultures. By their mathematical model they succeeded in explaining well the penicillin and streptomycin yields in batch and continuous cultures. In Pirt's approach *(4)*, the value of specific growth rate is relevant for product formation rate. Fishman and Biryukov developed the mathematical model for the process of penicillin production *(5)*. In the model, kinetics of growth, substrate uptake

* Reported at »Workshop on Biotechnology of Antibiotics, Steroids and Alkaloids of Medicinal Importance«, Sept. 1-5, 1986, Razgrad, Bulgaria.

and penicillin production as well as kinetics of synthesis of inhibitory metabolites are described by appropriate differential equations based on the assumption that the specific rate of penicillin bioynthesis is a function of the mean cumulative age of the culture. Authors applied this model in computing the optimal profile of glucose addition for attaining the higher yield in penicillin. Constantinides and Rai *(6)* in their model described growth kinetics by well-known logistic equation, whereas the modified Luedeking-Piret equation which includes a penicillin destruction term, was applied to describe kinetics of penicillin synthesis. On the basis of this model the optimum temperature profile for improved penicillin yield was calculated. Pontryagin's theorem *(7)* was applied by both groups of authors to calculate optimal profiles. In the model developed by Bajpai and Reuss *(8)* more factors relevant for the process course (e. g. dilution rate, microorganism maintenance, oxygen uptake and transfer rates) have been taken into account and a more complete model resulted. A good agreement of theoretical with the literature experimental data was found. Calam et al. *(9)* studied the process of griseofulvin biosynthesis. The mathematical model they applied was based on main biochemical reactions of oxidative conversion of substrates (carbon and nitrogen sources) into products (CO_2, antibiotic and ATP). The fit of experimental with computer simulation data was good. Process of erythromycin biosynthesis was also studied by applying mathematical models. The simple mathematical model developed on the basis of the threedimensional growth concept was successfully applied in explaining experimental growth, microorganism segregation, substrate uptake and erythromycin biosynthesis kinetics *(10,11)*. The same, completed with appropriate additional differential equations, described well kinetics of n-propanol evaporation and uptake *(12)*. The other concepts of expressing the growth kinetics were also analyzed and an appropriate model developed to find optimal pH and temperature profiles for improved erythromycin biosynthesis *(13)*. Marked attention was paid to the study of biosynthesis of tetracycline antibiotics. It was established that the equation expressing a threedimensional growth kinetics could be applied *(14)*. A structured kinetic model taking into account the relevant biochemical reactions was proposed to explain possible control mechanisms in tetracycline production *(15)*. There is no evidence of exact evaluation of this model by comparing theoretical with experimental data. The behaviour of microbial population and kinetics of process events during the process of oxytetracycline biosynthesis were analyzed by applying another structured model *(16)*. The model appeared to be useful in explaining qualitatively the process events, but it also suffered a default of quantitative support with experimental data. Simple mathematical model was used for evaluation of pH influence on *Streptomyces aureofaciens* growth and tetracycline biosynthesis *(17)*. Equations of first order turned out to be suitable in describing autolysis kinetics of streptomycetes *(16,18)*. In addition to the mentioned structured model *(16)* a more appropriate simple model was developed to describe the process of oxytetracycline biosynthesis. Validity of the model was confirmed by experimental batch and continuous culture data *(11,19-21)*. The aim of this work is to demonstrate the convenience of this model in explaining experimental results and in predicting the consequences of changes of cultivation conditions. Another aim is to show how the proposed model can be used in finding the way of improved oxytetracycline production and in improving the method of microorganism selection.

Methods

Experimental data

For the purpose of this work, recent, already published, our own experimental data *(10,11,20,21)*, as well as new experimental data were used. Data relate to experiments on laboratory, pilot-plant and plant scales.

Computer simulation

Digital computer simulations were performed. The computer »Hewlett-Packard-9845 B« was used.

Mathematical model

A. System of submerse culture

Growth kinetics

There is evidence for the growth kinetics of mycelial microorganisms that it could be expressed by the equation *(10,11,14,19-20)*

$$dx/dt = k_1 \cdot x^{2/3} - k_2 \cdot x \qquad /1/$$

or, for the higher range of batch processes by equations

$$dx/dt = k_1 \cdot x^{2/3} - k_2 \cdot x - k_3 \cdot x \cdot t \qquad /2/$$

or

$$dx/dt = k_1 \cdot x^{2/3} - k_2 \cdot x - k'_3 \cdot p \qquad /3/$$

If the continuous or repeated fed batch culture is applied, then the equation

$$dx/dt = k_1 \cdot x^{2/3} - k_2 \cdot x - k_3 \cdot x - x / (k_{vi} + (t - t_{i-1})) \qquad /4/$$

can be used *(11, 19-21)*.

Kinetics of substrate uptake

Growth kinetics is undoubtedly influenced by microorganism environment, i. e. by different substrates and other environmental factors. Kinetics of microbial utilization of any substrate can be the subject of study, but the media, where one substrate appears to be the process limiting factor can commonly be applied. Carbohydrates are precursors of tetracycline antibiotics and kinetics of their utilization appears to be relevant. In modelling carbohydrate uptake kinetics, one can usually recommend to distinguish the amount of carbohydrates used for microorganism maintenance and that used for growth, but more simple approach can also be applied. As recently demonstrated *(20, 21)*, the equation

$$ds/dt = (s_0 - s) / (k_{vi} + (t - t_{i-1}) - k_{sub} \cdot x \cdot s / (\alpha + s) \qquad /5/$$

showed to be a suitable one.

Kinetics of antibiotic synthesis:

Product formation kinetics in general could be represented by equation /6/

$$dp/dt = k_{p1} \cdot x - k_{p2} \cdot p + k_{p3} \cdot dx/dt \qquad /6/$$

but the use of simpler equation

$$dp/dt = k_p \cdot x \qquad /7/$$

was verified to be satisfactory *(11,21)*, if a limited range of cultivation conditions was considered. However, in larger range of cultivation conditions better agreement with experimental data was observed when the equation

$$dp/dt = k_{pm} \cdot x \cdot s / (s + \alpha) \qquad /8/$$

was applied *(21)*. Therefore, a general equation

$$dp/dt = k_{pm} \cdot x \cdot s / (s + \alpha) - p / (k_{vi} + t - t_{i-1}) \qquad /9/$$

appears to be convenient for expressing antibiotic synthesis kinetics in a batch as well as in continuous and repeated fed-batch cultures.

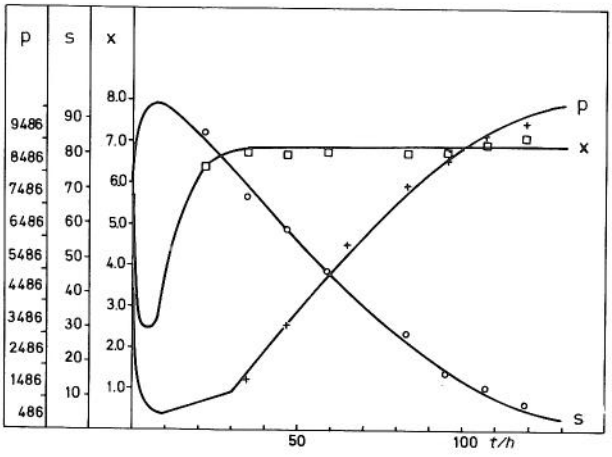

Fig. 1. Experimental and simulated oxytetracycline biosynthesis process course. Abscissa is cultivation time; the ordinates are (x) concentration of biomass expressed as autolysis phosphorus (mg/100 mL), (s) concentration of total carbohydrates (g/L) and (p) concentration of oxytetracycline (normalized values). Curves represent computer simulation data, while the points are experimental data. Constants: $k_1 = 0.93296$, $k_2 = 0.49$, $k_{pm} = 7.0$ (first 8 hours) and 24.7 (later period), $k_{sub} = 0.195$, $k_v = 0.784$ (first 8 hours) and 10^8 (later period), $s_0 = 101.0$, $\alpha = 20.0$.

Slika 1. Eksperimentalni i simulirani tijek procesa biosinteze oksitetraciklina. Apscisa je vrijeme uzgoja; ordinate su (x) koncentracija biomase izražena kao autolizni fosfor (mg/100 mL), (s) koncentracija ukupnih ugljikohidrata (g/L) i (p) koncentracija oksitetraciklina (normalizirane vrijednosti). Krivulje predstavljaju podatke kompjutorske simulacije, a točke su eksperimentalni podaci. Konstante $k_1 = 0.93296$, $k_2 = 0.49$, $k_{pm} = 7.0$ (prvih 8 sati) i 24.7 (kasnije razdoblje), $k_{sub} = 0.195$, $k_v = 0.784$ (prvih osam sati) i 10^8 (kasnije razdoblje), $s_0 = 101.0$, $\alpha = 20.0$.

B. System of microbial colonies

It was established *(22)* that the increase in colony size could be defined by equation

$$dD_c/dt = k_{1D} - k_{2D} \cdot D_c \qquad /10/$$

whereas the equation

$$dD_z/dt = q_{D2} \cdot D_c^2 / D_z \qquad /11/$$

or

$$dD_z/dt = q_{D3} \cdot D_c^3 / D_z \qquad /12/$$

can be used to define kinetics of inhibition zone increase. It was observed that particular isolates differ by their kinetic parameters. Potency index

$$I_p = D_z / D_c \qquad /13/$$

was proposed to be used as an appropriate strain selection criterion *(23)*.

Results

The available experimental data of one batch were analyzed by fitting computer simulation to experimental data and the result is presented in Fig. 1. Simulation constants found to be appropriate for the explanation of experimental values were used to simulate batches with larger inoculum size, slower medium addition rate and higher biomass and carbohydrates concentrations. Computer simulation results are presented in Fig. 2 and Table 1. The effects of temperature on process kinetics were also con-

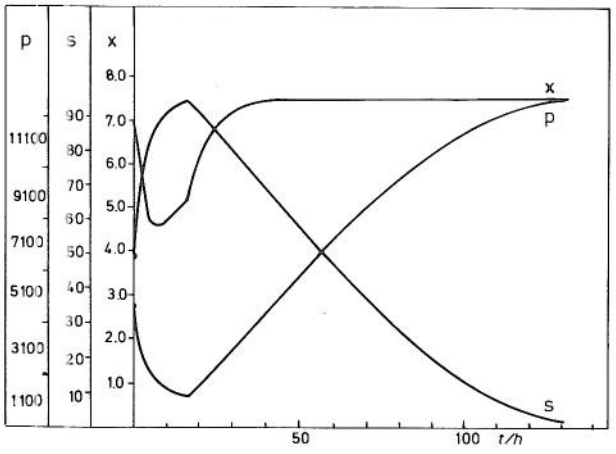

Fig. 2. Effects of slower medium addition rate and increased biomass and substrate concentrations on oxytetracycline biosynthesis. Computer simulation data. Symbols as in Fig. 1. Constants: $k_1 = 0.96$, $k_2 = 0.49$, $k_{pm} = 7.0$ (first 16 hours) and 24.7 (later period), $k_{sub} = 0.195$, $k_v = 4.0$ (first 16 hours) and 10^8 (later period), $s_0 = 115.0$, $\alpha = 20.0$.

Slika 2. Utjecaj sporije brzine dodavanja hranjive podloge i povećanih koncentracija biomase i supstrata na biosintezu oksitetraciklina. Podaci kompjutorske simulacije. Simboli kao na sl. 1. Konstante: $k_1 = 0.96$, $k_2 = 0.49$, $k_{pm} = 7.0$ (prvih 16 sati i 24.7 (kasnije razdoblje), $k_{sub} = 0.195$, $k_v = 4.0$ (prvih 16 sati) i 10^8 (kasnije razdoblje), $s_0 = 115.0$, $\alpha = 20.0$.

Table 1. Simulated oxytetracycline yields at the 100th hour of cultivation. Initial conditions: x_{in} = 8.54, s_{in} = 49.44, p_{in} = 4670, s_0 = 101, α = 20*; x_{in} = 451.5, s_{in} = 53.1, p_{in} = 4600, s_0 = 105, α = 10**.
Tablica 1. Simulirani prinosi oksitetraciklina u 100. satu uzgoja. Početni uvjeti: x_{in} = 8.54, s_{in} = 49.44, p_{in} =4670, s_0 = 101, α = 20*; x_{in}= 451.5, s_{in}= 53.1, p_{in} = 4600, s_0 = 105, α = 10**.

Time Vrijeme h	Values of constants Vrijednosti konstanti					Yield Prinos
	k_1	k_2	k_v	k_{sub}	k_p	
0-8	0.93296	0.49	1.0	0.195	7.0	*
8-29	0.93296	0.49	10^8	0.195	7.0	
29-100	0.93296	0.49	10^8	0.195	24.7	8934
0-7	0.93296	0.49	2.0	0.195	7.0	*
7-21	0.93296	0.49	10^8	0.195	7.0	
21-100	0.93296	0.49	10^8	0.195	24.7	9750
0-9	0.820	0.10	1.0	0.0021	0.231	**
9-24	0.820	0.10	10^8	0.0021	0.231	
24-100	1.476	0.18	10^8	0.0026	0.286	9285
0-8	0.820	0.10	2.0	0.0021	0.231	**
8-24	0.820	0.10	10^8	0.0021	0.231	
24-100	1.476	0.18	10^8	0.0026	0.286	9947
0-8	0.820	0.10	2.0	0.0021	0.231	**
8-24	0.820	0.10	10^8	0.0021	0.231	
24-100	1.722	0.21	10^8	0.0028	0.308	10465
0-16	1.800	0.21	4.0	0.0028	0.308	**
16-100	1.800	0.21	10^8	0.0028	0.308	11214

* Biomass evaluted as autolysis phosphorus; Biomasa vrednovana kao autolizni fosfor
** Biomass evaluated as 2,6-L,L-diaminopimelic acid (DAPA); Biomasa vrednovana kao 2,6-L-L-diaminopimelinska kiselina (DAPA)

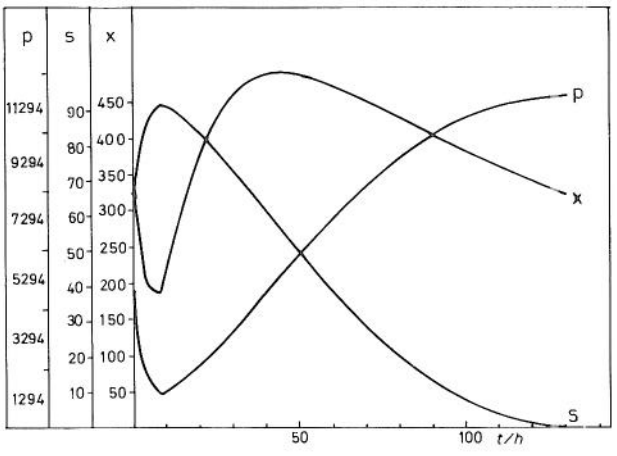

Fig. 3. Computer simulation of the process course of oxytetracycline biosynthesis at higher cultivation temperature and more evident biomass autolysis. Biomass evaluated as DAPA. Symbols as in Fig. 1. Constants: for the first 8 hours: k_1 = 1.8, k_2 = 0.203, k_3 = 0.00045, k_v = 2.0, other as in later period; later period: k_1 = 1.76, k_2 = 0.203, k_3 = 0.00045, k_{pm} = 0.308, k_{sub} = 0.0028, k_v = 108, s_0 = 105.0, α = 10.0.
Slika 3. Kompjutorska simulacija tijeka procesa biosinteze oksitetraciklina pri višoj temperaturi uzgoja i pri očitijoj autolizi biomase. Biomasa vrednovana kao DAPA. Simboli kao na sl. 1. Konstante: za prvih 8 sati: k_1 = 1.8, k_2 = 0.203, k_3 = 0.00045, k_v = 2.0, ostalo kao u kasnijem razdoblju; kasnije razdoblje: k_1 = 1.76, k_2 = 0.203, k_3 = 0.00045, k_{pm} = 0.308, k_{sub} = 0.0028, k_v = 108, s_0 = 105.0, α = 10.0.

sidered. Computer simulation constants were chosen according to the previous work *(21)* and the simulations were performed in order to predict the culture behaviour at lower and higher cultivation temperatures, taking into account the effect of inoculum size and medium addition as well. Some results are shown in Table 1.

Fig. 3 gives detailed insight into the course of one of the simulated processes. In Table 2 some relevant experimental data are presented.

To demonstrate further applications of presented mathematical model, the process of erythromycin biosynthesis was also simulated. As in the case of some examples of oxytetracycline biosynthesis, the applied values of kinetic constants were chosen to be similar to those previously established as appropriate ones. Some of information on the results of simulation is summarized in Table 3, while more information, on the one of possible cases is given in Fig. 4.

Growth kinetics of microbial colonies of *S. rimosus* R6 isolates and their kinetics of oxytetracycline biosynthesis were also considered. In Fig. 5, the simulated potency index values for two typical *S. rimosus* R6 isolates are shown. Finally, Table 4 presents summarized data illustrating the application area of presented mathematical model.

Discussion

It is evident from Fig. 1 that the applied mathematical model is a convenient one. Computer simulation data fit well to the experimental ones. It follows that the estimated values of kinetic constants could be applied for different purposes if the application is in the range where the behavior of the real system can be well explained by estimated values of constants. When data in Fig. 1 are

Table 2. Experimental oxytetracycline yields (normalized values). Abbreviations: SI = small inoculum, LI = large inoculum, LT = lower cultivation temperature and HT = higher cultivation temperature applied.

Tablica 2. Eksperimentalni prinosi oksitetraciklina (normalizirane vrijednosti). Kratice: SI = malo cjepivo, LI = veliko cjepivo, LT = niža temperatura uzgoja i HT = viša temperatura uzgoja.

Ferment. group / Ferment. skupina	Inoculum size / Veličina cjepiva %	Fed-batch period / Razdoblje prihranjivanja h	Antibiotic yield / Prinos antibiotika average / prosjek	st. dev. / st. dev.	Cultivation time / Vrijeme uzgoja average / prosjek	st. dev. / st. dev.
SI – LT	10	0-9	(4) 100.0	3.3	121.7	11.4
SI – HT	10	0-9	(7) 108.3	3.6	116.7	6.0
LI – LT	20	0-8	(8) 111.1	4.9	107.6	7.4
LI – HT	20	0-8	(5) 114.2	1.9	106.5	9.7
the best LI – LT	20	0-8	106.5	0	91	0
the best LI – LT	20	0-8	107.6	0	81	0

Remark: Number in brackets refers to the number of batches in the group
Napomena: Broj u zagradi označuje broj šarži u skupini

analyzed more precisely it becomes obvious that the specific rate of oxytetracycline biosynthesis is slower during the growth than the stationary or production phase. This phenomenon can partly be explained by difference in cultivation temperature (data are not presented here), because it was lower during the first 30 hours than in the later period, but other reasons cannot be neglected too. The biomass concentration was evaluated as autolysis phosphorus. In such a way estimated values probably do not always represent the amounts of biomass of the age appropriate for oxytetracycline biosynthesis. Since the same values can well explain the carbohydrate uptake and heat evolution kinetics *(20)*, it could be considered that the autolysis phosphorus represents biomass pool (potency) rather with respect to reactions of primary than secondary metabolism. Growth is fast during the fed-batch phase and for a relatively short period following that phase. The mean commulative age of the biomass is therefore relatively low during the growth period. As a consequence, the lower specific oxytetracycline biosynthesis rates result. Moreover, it is well known that the presence of phosphates in the medium is favourable for growth and not for oxytetracycline biosynthesis. When well balanced media are used, the phosphates are usually exhausted from the medium when the microorganism enters the stationary, i. e. the production phase. These considerations permit the assumption that shorter periods of lower specific oxytetracycline biosynthesis rate occur in cases where larger inocula and slower medium addition rates would be applied. If such a hypothesis is correct then the results as those in Table 1 and Fig. 2 could be expected. It is evident that higher yields could be attained faster if larger inocula are applied. Another method of biomass evaluation was by 2,6-L,L-diaminopimelic acid (DAPA). For a constant cultivation temperature a good agreement of experimental with computer simulation data was observed, even when constant k_{sub} and k_p values have been applied. Temperature increase induced faster process kinetics and resulting higher values of kinetic constants *(21)*. In this work a series of computer simula-

Table 3. Simulated erythromycin yields upon 180 hours of cultivation. Biomass evaluted as 2,6-L-L-diaminopimelic acid. Initial conditions: $x_i = 50.0$, $s_i = 15.0$, $p_i = 500.0$, $s_0 = 30$, $\alpha = 10$ (first example); $x_i = 100$, $s_i = 30.0$, $p_i = 1000$, $s_0 = 60$, $\alpha = 10$ (other examples).

Tablica 3. Simulirani prinosi eritromicina nakon 180 sati uzgoja. Biomasa vrednovana kao 2,6-L-L-diaminopimelinska kiselina. Početni uvjeti: $x_i = 50.0$, $s_i = 15.0$, $p_i = 500.0$, $s_0 = 30$, $\alpha = 10$ (prvi primjer); $x_i = 100$, $s_i = 30.0$, $p_i = 1000$, $s_0 = 60$, $\alpha = 10$ (ostali primjeri).

Time / Vrijeme h	Values of constants / Vrijednosti konstanti k_1	k_2	$k_3 \times 10^5$	k_v	$k_{sub} \times 10^3$	k_p	Yield / Prinos
0-8	0.330	0.064	0	2.0	2.8	0.251	*117
8-180	0.330	0.064	17	10^8	2.8	0.251	1678
0-8	0.530	0.090	0	2.0	2.8	0.251	*240
8-180	0.531	0.090	0	10^8	2.8	0.251	4740
0-9	0.530	0.090	0	1.0	2.8	0.251	*126
9-180	0.531	0.090	0	10^8	2.8	0.251	4728
0-9	0.470	0.070	0	1.0	1.8	0.160	*117
9-180	0.469	0.070	30	10^8	1.8	0.160	2242
0-8	0.530	0.090	0	2.0	1.45	0.130	*221
8-180	0.531	0.090	0	10^8	1.45	0.130	3039

* data relate to the end of fed-batch period; podaci se odnose na kraj razdoblja prihranjivanja

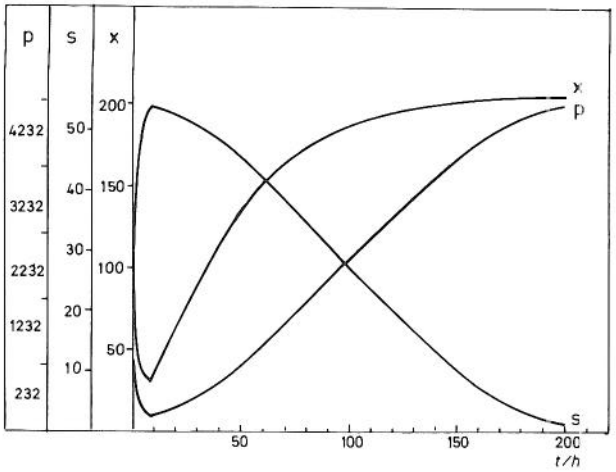

Fig. 4. Computer simulation of erhythromycin biosynthesis process. Biomass evaluated as DAPA. The abscisa is cultivation time (h), the ordinates are (x) biomass concentration, (s) carbohydrate concentration and (p) erhythromycin concentration. Constants: $k_1 = 0.53$, $k_2 = 0.09$, $k_{pm} = 0.251$, $k_{sub} = 0.0028$, $k_v = 2.0$ (first 8 hours) and 108 (later period), $s_0 = 60.0$, $\alpha = 10.0$.

Slika 4. Kompjutorska simulacija procesa biosinteze eritromicina. Biomasa vrednovana kao DAPA. Apscisa je vrijeme uzgoja (h), ordinate su (x) koncentracija biomase, (s) koncentracija ugljikohidrata i (p) koncentracija eritromicina. Konstante: $k_1 = 0.53$, $k_2 = 0.09$, $k_{pm} = 0.251$, $k_{sub} = 0.0028$, $k_v = 2.0$ (prvih 8 sati) and 108 (kasnije razdoblje), $s_0 = 60.0$, $\alpha = 10.0$.

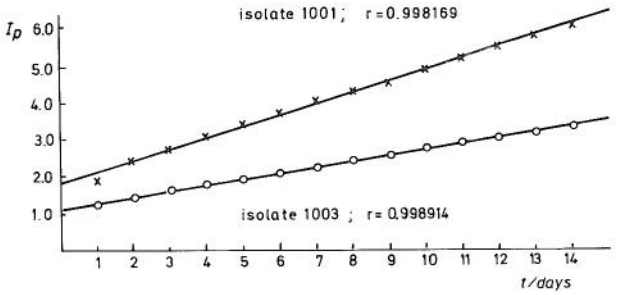

Fig. 5. Simulated potency index values for two typical *S. rimosus* R6 isolates. The abscisa is cultivation time, the ordinate potency index Ip. Constants: Isolate 1001: $k_{1D} = 2.75$, $k_{2D} = 0.2$, $q_{D2} = 2.0$; Isolate 1003: $k_{1D} = 3.62$, $k_{2D} = 0.15$, $q_{D2} = 0.66$.

Slika 5. Simulirane vrijednosti indeksa sposobnosti dvaju tipičnih izolata bakterije *S. rimosus* R6. Apscisa je vrijeme uzgoja (dani), ordinata je indeks sposobnosti Ip. Konstante: Izolat 1001: $k_{1D} = 2.75$, $k_{2D} = 0.2$, $q_{D2} = 2.0$; izolat 1003: $k_{1D} = 3.62$, $k_{2D} = 0.15$, $q_{D2} = 0.66$.

tions has been performed by applying simulation kinetic constants already found to be appropriate. As it can be observed in Table 1, increase in temperature and inoculum size appear to be favourable for oxytetracycline biosynthesis. Experimental data shown in Table 2 support such a conclusion. If cultivation conditions become unfavourable for growth, inducing microbial cell death and autolysis, then the result as in Fig. 3 could be more frequent, because by pH and mass transfer control as well as by addition of corresponding substrate, biomass death

Table 4. Model applications
Tablica 4. Primjena modela

Subject of the study Predmet proučavanja	Purpose and results Svrha i rezultati	References Referencije
Process of oxytetracycline biosynthesis	Description of growth, substrate uptake, antibiotic synthesis and heat evolution kinetics; explanation of amylase secretion kinetics. Prediction of process course. Distinguishing of microbiol. isolates; colony growth kinetics (Report at the Symposium – GIM, 86, Split, Croatia)	*14,11,19-21, 24, 19,25, 22,*
Proces biosinteze oksitetraciklina	Opis kinetike rasta, potrošnje supstrata, sinteze antibiotika i razvijanja topline; objašnjenje kinetike sekrecije amilaze. Pretkazivanje tijeka procesa. Razlikovanje mikrobnih izolata; kinetika rasta kolonija (izvješće na: Simpozium GIM 86, Split, Croatia)	*14,11,19-21, 24, 19,25, 22,*
Proces of erythromycin biosynthesis	Description of process kinetics; microorganism segregation, precursor evaporation and uptake; prediction of results (with additional equations)	*9-12, 25,*
Proces biosinteze eritromicina	Opis procesne kinetike; segregacija mikroorganizama, isparivanje i potrošnja prekurzora; pretkazivanje rezultata	*9-12 25,*
Process of glucoamylase production	Description of process kinetics (including mycovirus propagation kinetics)	*26,*
Proces proizvodnje glukoamilaze	Opis procesne kinetike (uključivo i kinetike propagacije mikovirusa)	*26,*
Glucose isomerase production	Description of growth kinetics	*27,*
Proizvodnja glukozne izomeraze	Opis kinetike rasta	*27,*
Growth of multicellular tumor spheroids (MTS)	Description of growth kinetics; explanation of MTS behaviour	*28,*
Rast višestaničnih tumorskih sferoida (MTS)	Opis kinetike rasta; objašnjenje ponašanja MTS	*28*

and autolysis rates can be retarded markedly. In such a way the inconvenient culture conditions can usually be avoided, or at least their appearance delayed.

When comparing the real systems perhaps one could consider the process of erythromycin biosynthesis to be more complex than that of oxytetracycline biosynthesis. However, the mathematical model was already verified as the convenient one for the description of the process of erythromycin biosynthesis *(11)*. Therefore, results like those simulated (Table 3 and Fig. 4) could really be expected to occur in real systems. Of course, some difficulties could appear in practical realization of the process course like that shown in Fig. 4. Control of pH value and biomass and substrate concentrations would probably be necessary, at least in the latest period.

Applications of the presented model are not strictly limited to processes of antibiotic biosynthesis. Data presented in Table 4 suggest that the application range is larger. Moreover, it should be pointed out that the model can be extended by adding new terms and/or equations, in order to apply it in describing more process events. Another possibility is to convert the model into other forms, e. g. into the model describing kinetics of events during the growth of microbial colonies. Indeed, very good agreement of experimental with computer simulation values of colony and inhibition zone diameter has been observed when equations /10/, /11/ and /12/ have been applied *(22)*. Data in Fig. 5 clearly demonstrate how the particular *S. rimosus* isolates could be distinguished on the basis of potency index values. In addition, data suggest that colony age is a very important factor which should be taken into account when defining the methods for screening microbial isolates. Finally, it is important to point out that the utility of mathematical modelling of biochemical systems, such as the system of synthesis of secondary metabolites, is not because of their adequate description and the possibility to simulate processes only. More important utility results from the fact that discrepances between theoretical and experimenthal data induce further investigations in the sense of finding the reasons for differences. Process and mathematical model improvements usually appear as a consequence of such investigations.

Conclusions

The presented mathematical model describes well the kinetics of main events during processes of antibiotics synthesis.

Within the range of its validity, the model can be successfully applied in predicting the microbial culture behaviour upon application of new cultivations.

The model appears to be useful in defining and improving the methods of screening microbial strains.

Application range of the presented mathematical model exceeds the field of antibiotic synthesis.

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Nomenclature

dD_c/dt = derivative of D_c with respect to t: rate of colony diameter increase (L/T)

dD_z/dt = derivative of D_z with respect to t: rate of inhibition zone diameter increase (L/T).

D_c = colony diameter (L)

dp/dt = derivative of p with respect to t: antibiotic synthesis rate or rate of change of antibiotic concentration ($M/L^3/T$)

ds/dt = derivative of s with respect to t: substrate uptake rate or rate of change of substrate concentration ($M/L^3/T$)

dx/dt = derivative of x with respect to t: growth rate or rate of change of biomass concentration ($M/L^3/T$)

D_z = inhibition zone diameter (L)

i = ordinal number of the cycle in repeated fed-batch culture (dimensionless)

I_p = potency index (dimensionless)

k_1 = cubic growth rate constant ($M^{1/3}/L/T$)

k_2 = constant of growth rate supression (T^{-1})

k_3 = constant of growth rate retardation (T^{-2})

k_{1D} = constant: maximal rate of colony diameter increase (L/T)

k_{2D} = constant of supression of colony diameter increase (T^{-1})

k_p = specific rate of antibiotic synthesis (T^{-1})

k_{p1} = constant of not growth associated product formation rate (T^{-1})

k_{p2} = constant of product (antibiotic) degradation rate (T^{-1})

k_{p3} = constant of growth associated product formation rate (dimensionless)

k_{pm} = maximal specific rate of antibiotic synthesis (T^{-1})

k_{sub} = maximal specific rate of substrate uptake (T^{-1})

k_{vi} = constant: reciprocal dilution rate at the start of fed-batch cycle (T)

L = length

M = mass

p = product (antibiotic) concentration (M/L^3)

q_{D2} = specific rate of inhibition zone linear increase (T^{-1})

q_{D3} = specific rate of inhibition zone linear increase ($L^{-1}T^{-1}$)

s = substrate concentration (carbohydrates) (M/L^3)

s_0 = substrate concentration in nutrient medium (M/L^3)

t = cultivation time (T)

t' = cultivation time at the end of fed-batch cycle (T)

T = time

x = biomass concentration (M/L^3)

Nazivlje

dD_c/dt = derivacija D_c u odnosu na t: brzina povećavanja promjera kolonije (L/T)

dD_z/dt = derivacija D_z u odnosu na t: brzina povećavanja promjera zone inhibicije (L/T)

D_c = promjer kolonije (L)

dp/dt = derivacija p u odnosu na t: brzina sinteze antibiotika ili brzina promjene koncentracije antibiotika ($M/L^3/T$)

ds/dt = derivacija s u odnosu na t: brzina potrošnje supstrata ili brzina promjene koncentracije supstrata ($M/L^3/T$)

dx/dt = derivacija x u odnosu na t: brzina rasta ili brzina promjene koncentracije biomase ($M/L^3/T$)

D_z = promjer zone inhibicije (L)

i = redni broj ciklusa u opetovanom prihranjivanom šaržnom uzgoju

I_p = indeks sposobnosti

k_1 = konstanta brzine kubičnog rastenja ($M^{1/3}/L/T$)

k_2 = konstanta kočenja rastenja (T^{-1})

k_3 = konstanta usporavanja rastenja (T^{-2})

k_{1D} = konstanta: maksimalna brzina povećavanja promjera kolonije (L/T)

k_{2D} = konstanta kočenja povećavanja promjera kolonije (L/T)

k_p = specifična brzina sinteze antibiotika (T^{-1})

k_{p1} = konstanta brzine uz rast nevezane tvorbe produkata (T^{-1})

k_{p2} = konstanta brzine razgradnje produkta (antibiotika) (T^{-1})

k_{p3} = konstanta brzine uz rast vezane tvorbe produkta

k_{pm} = maksimalna specifična brzina sinteze antibiotika (T^{-1})

k_{sub} = maksimalna specifična brzina potrošnje supstrata (T^{-1})

k_{vi} = konstanta: recipročna brzina razrjeđivanja pri početku ciklusa prihranjivanja šarže (T)

L = duljina

M = masa

p = koncentracija produkata (antibiotika) (M/L^3)

q_{D2} = specifična brzina linearnog povećavanja zone inhibicije (T^{-1})

q_{D3} = specifična brzina linearnog povećavanja zone inhibicije ($L^{-1}T^{-1}$)

s = koncentracija supstrata (M/L^3)

s_0 = koncentracija supstrata u čistoj hranjivoj podlozi (M/L^3)

t = vrijeme uzgoja (T)

t' = vrijeme uzgoja pri kraju ciklusa šaržnog prihranjivanja (T)

T = vrijeme

x = koncentracije biomase (M/L^3)

Greek letters

α = constant: substrate concentration where specific rate of substrate uptake is one half of its maximal value (M/L^3)

Grčka slova

α = konstanta: koncentracija supstrata kada je specifična brzina potrošnje supstrata jednaka polovici maksimalne vrijednosti (M/L^3)

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