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Introduction to the Theoretical Fundamentals for Increasing the Probability of Heterologous Contacts between Biological Particles

Marijan Bošnjak and Stjepan Bogdan***

Faculty of Food Technology and Biotechnology,
Pierottijeva 6, P.O.B. 625, HR-10000 Zagreb, Croatia

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Dedicated to the memory of Professor Vera Johanides

Summary

An introduction to the theoretical fundamentals referring to the contacts appearing between biological particles is given. Factors affecting the probable frequency of different contacts were analysed with reference to their effects on possible heterologous recombinations which could lead to the appearance and the growth of microbial cells having new properties and being capable to produce new biologically active compounds. Effects of the differences in bioparticle morphology and cultivation methods are discussed. Kinetics of the process events in hypothetical mixed culture of the two parent microbial strains were studied applying computer simulation. This was performed on the basis of mathematical model developed for such a purpose. Results of computer simulation suggest that the applied mathematical model appears to be the adequate one for explaining the growth kinetics of a new strain and its parent strains. The kinetics of other events can also be well explained. In discussing the relevance of particular parameters a special emphasis was given to the biological character of microbial cells. The ability of microbial cells to adapt their properties and behaviour in accordance to the properties of their environment is a factor of essential importance.

Key words: bioparticle contact probability, recombinations, morphology effects, mathematical analysis, kinetics, computer simulation, batch and continuous culture

Introductory Consideration

The contact, or even crash, between two particles or reagents is the prerequisite for a reaction between them regardless of their nature, *i.e.* one can consider that the reaction is possible only after the contact between two mass particles or between mass particle and some agent of undulatory character, like UV- and X-rays, is realised. It is known that the simultaneous contact between three simple molecules is of very low probability, and therefore there is no need to consider such cases here. Of course, we should bear in mind that there is a difference between various contacts, which commonly depends on particle size and form as well as on the action of catching or repulsive forces existing in the reaction system. In systems where particles largely vary in their size the simultaneous multiparticle contacts become more probable. Basically, the chemical reaction systems are well de-

* Corresponding author

** Hobby participation; permanent employment address: PLIVA d.d., Prilaz baruna Filipovića 25, HR-10000 Zagreb, Croatia

fined with respect to their kinetics, although even among such systems we should distinguish those ideal from the nonideal ones. For ideal systems, *i.e.* ideal gases and ideal solutions, the thermodynamic equations are greatly simplified *(1)*, because of the absence of intermolecular forces in ideal gases and the uniformity of such forces in ideal solutions. Real gases and real solutions deviate from ideality and new functions were introduced to express deviations from ideality as well as to define both systems by common mathematical model. Thus, for a gas, an ideal partial pressure instead of particular pressure the function called the *fugacity*, was introduced while for a component in solution, instead of concentration or mole fraction, a function called the *activity* was introduced *(1)*. In real systems we should also consider the differences between gases, crystals and liquids. Because the extremes of total chaos and perfect order are both relatively simple to treat mathematically, the theories of gases and crystals (solids) are well advanced, while in the case of liquids the situation is much harder to define. The liquid state, a compromise between order and disorder, has so far defined comprehensive theoretical treatment *(1)*. However, despite the complexity of nonideal systems an oversimplification can be applied when describing such systems mathematically. Therefore, we can start with a rough analysis of the reaction system where molecules of one chemical compound (A) react with those of another (B), giving the reaction product (C). Such a reaction system can be schematically presented as

$$A+B \xrightarrow{k_1} C \qquad /1/$$

whereas for its reaction kinetics the equations

$$dC_C/dt = -dC_A/dt = -dC_B/dt = k_1 \cdot C_A \cdot C_B \qquad /2/$$

can be applied, if C_A, C_B and C_C are the concentrations of reaction substances A, B and C, while k_1 = reaction rate constant and t = reaction time. The maximal reaction rates can be expected when reactants are present in equimolar concentrations. In a given reaction mixture the contacts between different particles are possible, but the product C appears as a consequence of the reaction between particles of compounds A and B. If the compound A cannot react with the compound B, then the compound C cannot be formed. Therefore, contacts between particles do not obligatorily cause nor lead to the appearance of product particles, since in addition to the contact, *i.e.* collision between particles, energetic and some other conditions should be fulfilled for a reaction to occur. In some more complex reaction system

$$A+B \underset{-k_2}{\overset{k_1}{\rightleftharpoons}} C+D \qquad /3/$$

where the equilibrium between reaction rates and therefore between concentrations of products and reagents is finally expected, the following system of equations can be applied

$$dC_A/dt = dC_B/dt = -k_1 \cdot C_A \cdot C_B + k_2 \cdot C_C \cdot C_D \qquad /4/$$

$$dC_C/dt = dC_D/dt = k_1 \cdot C_A \cdot C_B - k_2 \cdot C_C \cdot C_D \qquad /5/$$

When the equilibrium is established, the relations

$$dC_A/dt = dC_B/dt = dC_C/dt = dC_D/dt = 0 \qquad /6/$$

$$(C_C \cdot C_D)/(C_A \cdot C_B) = k_1/k_2 = K \qquad /7/$$

can be applied. As demonstrated by equations /4–7/, the contacts between particles of reaction products can also be productive, giving the starting reagents by an opposite reaction. It is important to point out that in equations /2/ and /4–7/ the values of kinetic constants can vary depending on deviations of the real system with respect to that ideal.

There is a series of much more complex chemical reaction systems. However, biochemical reaction systems, especially those referring to the live organisms or their cells, certainly can be and commonly are of even extremely higher complexity. This fact should be taken into account when studying the biochemical reaction systems and therefore when evaluating the relevance of contacts between different cells, microorganisms and/or biologically important chemical substances present in the environment of cells or organisms. Disregarding the degree of complexity there is a lot in common between catalytic chemical reaction systems and biochemical reaction systems *(1,2)*. Moreover, similar approach can be applied when describing mathematically the kinetics of chemical reaction systems and biochemical reactions *in vitro*, pharmacokinetics, kinetics of microbial processes *(2,3)* and even kinetics of very complex ecological systems *(3,4)*. The aim of this study is to analyse the systems of microbial populations with respect to the frequency of probable contacts between particular cells or cell agglomerates, with a special emphasis on the frequency of probable contacts between cells differing in genetic properties.

Different methods of transferring genetic materials from one cell to another or from one organism to another can be applied. The intention is to produce cells or organisms with new genetic properties. Cell recombinations are normal phenomena which can result in a formation of cells or organisms with changed or new properties. However, recent advances in the theoretical knowledge and experimental techniques enabled successful recombinations even between cells showing lower degree of homology *(5)*. To increase the experimental efficiency of heterologous recombinations the effects of all relevant factors should be analysed. Majority of them can be classified and described as follows:

1) factors affecting cell wall and cell membrane permeability for agents that can enter the cell and then act on genetic material as mediators or compounds capable to modify a genetic structure; key factors responsible for »cell door« opening and »cell door« adaptation,

2) factors being directly responsible for changes of the genetic material, acting as mediators (vectors), as compounds modifying DNA structure by affecting the chemical nature of particular units or sequences, or as DNA sequences which can be inserted into DNA structure, *etc.*,

3) factors affecting cell morphology and cell agglomeration, and therefore the size and compatibility of biological particles, especially relevant for filamentous and pellet forming microorganisms,

4) factors affecting the rate of approaching the cells to the critical distance between them, or enabling the

contacts between particular cells or particles, *i.e.* factors affecting the expression of specific activities of factors belonging to the groups 1) and 2).

Our actual intention is to focus our present scientific interest on the factors of the group 4). We decided to apply the logics already established as valid in interpreting the kinetics of chemical reaction systems. Since, with respect to oxygen supply techniques, the aerobic microbial cultures can be classified into two types:

a) submerse microbial cultures where microorganisms are cultivated in aerated and agitated liquid nutrient media, and

b) surface microbial cultures where microorganisms, exposed to the air, grow on surfaces of solid nutrient media, each of the mentioned two types should be analysed separately.

Behaviour of Microorganisms in Submerse Cultures

When an aim is to apply submerse microbial cultures in order to induce more frequent recombinations between microbial cells of different genetic properties (heterologous recombinations), it is recommended primarily to prepare the mixture of suspended cells of two different strains (or of their genetically relevant equivalents). Then, the reaction mixture containing the suspended particles representing either particular cells or agglomerates containing a number of cells, should be subjected to the cultivation process. Since during the cultivation process the continuous aeration and agitation are applied, one can expect the particles with microbial cells to be distributed homogeneously over the whole culture volume. The most simple cases are those referring to the population mixture of two cell kinds and/or cell agglomerates of the same size and shape. If the simultaneous contacts between three cells or three particles can be considered to happen with extremely low probability, then the evaluation of probabilities for two-particle contacts or crashes can be simplified, *i.e.* the appearance of the simultaneous three-particle crashes can be neglected (although such contacts cannot be excluded). In the microbial culture of two genetically diverse strains »A« and »B« three types of contacts are possible: A-A, B-B and A-B. Their frequency is expected to be the function of the number of particles, their affinities for particular contacts and the culture medium properties (like medium viscosity, concentration of different substances, *etc.*), *i.e.* it can be considered that

$$f_{A\text{-}A} = q_1 \cdot N_A \cdot N_A = q_1 \cdot N_A^2 \qquad /8/$$

$$f_{B\text{-}B} = q_2 \cdot N_B \cdot N_B = q_2 \cdot N_B^2 \qquad /9/$$

$$f_{A\text{-}B} = q_3 \cdot N_A \cdot N_B \qquad /10/.$$

From the point of view of heterologous recombinations the value of $f_{A\text{-}B}$ appears to be the most relevant. Its probability coefficient, $P_{f(A\text{-}B)}$, could be defined as follows:

$$P_{f(A\text{-}B)} = q_3 \cdot N_A \cdot N_B / (q_1 \cdot N_A^2 + q_2 \cdot N_B^2 + q_3 \cdot N_A \cdot N_B) \qquad /11/.$$

As shown by equations /8–11/ the values of frequency factors and probability coefficients depend on numbers of particular particles. Distribution of relative values vary depending on the participation of particular strains in the population mixture. Calculated data are presented in Fig. 1.

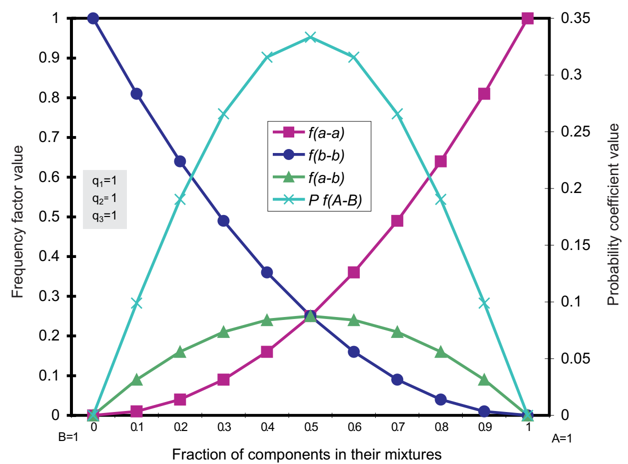


Fig. 1. Effect of particle participation on values of frequency factors and probability coefficients for $q_1 = q_2 = q_3 = 1$

Data clearly show that the optimal values for heterologous contacts could be expected in such a population mixture where a relation between numbers of strain particles can be expressed as

$$N_A : N_B = 1 : 1 \qquad /12/.$$

Cell affinity for other cells, cell structure obstacles and cell physiological state affect the values of kinetic constants. The effects of differences in proportionality factor values are demonstrated in Fig. 2 and Fig. 3. If particles represent individual cells or agglomerates of extremely small size (*e.g.* $V_{agl} \leq 10$ µm), the calculated data could be taken as a reliable argument for the conclusion that in such cases the factors of the group 4) do not limit sinificantly the efficiency of heterologous

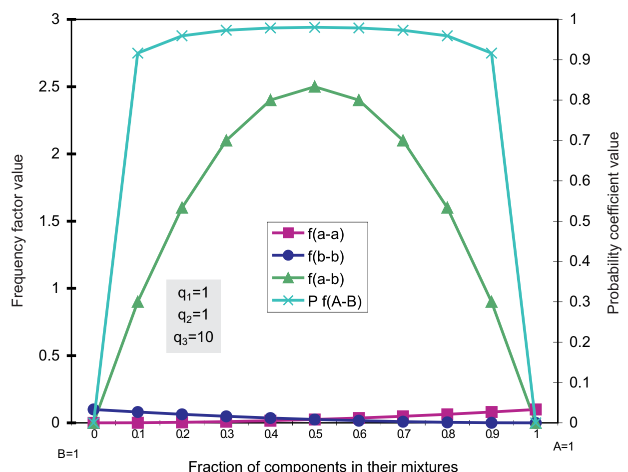


Fig. 2. Effect of particle participation on values of frequency factors and probability coefficients for $q_1 = q_2 = 0.1\ q_3 = 1$

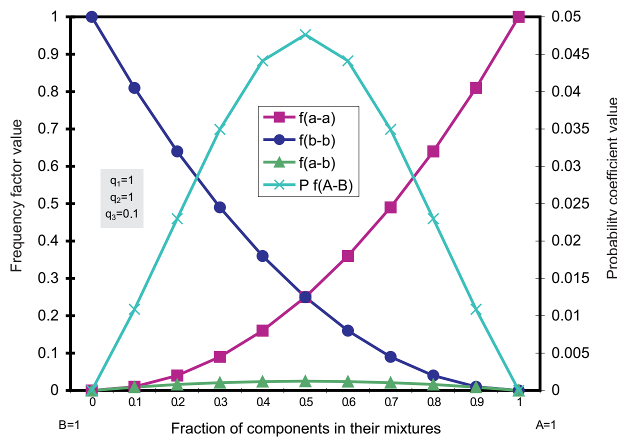


Fig. 3. Effect of particle participation on values of frequency factors and probability coefficients for $q_1 = q_2 = 10\, q_3 = 1$

recombinations, *(i)* if the concentration of any member of the population mixture is not too low, and *(ii)* if the affinity for heterologous contacts is expressed enough. One can also consider that in such cases the factors of the group 3) either led to the given relations or did not have a significant influence. The situation becomes much more complex in cases where much larger agglomerates are formed. Then, practically there is no limitation for homologous contacts because the agglomerated cells are in a continuous internal contact. Quite contrary, the probability for heterologous cell contacts then becomes extremely decreased, since only the cells occupying the external layers of particular agglomerates have a possibility to arrive in a position for crashing with cells of other agglomerates. This is especially pronounced in cases of dense and compact packing of cell agglomerates.

Data in Table 1 demonstrate the variation of participation of outer layer cells with respect to the total number of cells in agglomerates of different size. The participation of agglomerate outer layer cells practically represents the ratio between agglomerate outer surface area and agglomerate total volume. Therefore, the ratio values change proportionally to the reciprocal values of agglomerate diameters, if the agglomerates are of spheroidal form. It is clear that the participation of cells which can be exposed to heterologous contacts decreases with agglomerate size increase. Based on this consideration we become capable to calculate the more adequate probability coefficients. In the simplest case of agglomerates of the same size and equal number of cells it can be taken that the total number of cells in particular agglomerate, n_{clag}, can be estimated by using the formula

$$n_{clag} = V_{agl} \,/\, v_{cell} \qquad /13/.$$

Then, the number of cells occupying the outer layer surface, n_{clol}, can be estimated on the basis of the expression

$$n_{clol} = S_{ola} \,/\, s_{cell} \qquad /14/.$$

It follows that the proportion of outer layer cells in the given agglomerate, n_f, can be calculated on the basis of expression

$$n_f = n_{clol} \,/\, n_{clag} \qquad /15/.$$

Therefore, the frequency factor of heterologous contacts, $f_{(A\text{-}B)nf}$, should be calculated after converting the equation /10/ into

$$f_{(A\text{-}B)nf} = q_3 \cdot N_A \cdot N_B \cdot n_{fa} \cdot n_{fb} \qquad /16/.$$

Accordingly, the probability coefficient for heterologous contacts, $P_{f(A\text{-}B)nf}$, should be calculated by using the modified expression

$$P_{f(A\text{-}B)nf} = f_{(A\text{-}B)nf} \,/\, (f_{(A\text{-}B)nf} + W_A \cdot f_{(A\text{-}A)} + W_B \cdot f_{(B\text{-}B)}) \quad /17/$$

in which W_A and W_B represent the weighting factors expressing the effects of continuous contacts between identical cells in particular agglomerate. W_A and W_B are the reflection of the total cell numbers in particular agglomerate, or more precisely, of the total number of viable cells in particular agglomerate. Since the values of weighting factors are supposedly larger than 1.0, and the value of $f_{(A\text{-}B)nf}$ is evidently inferior to either $f_{(A\text{-}A)}$ or $f_{(B\text{-}B)}$, the value of the probability coefficient, $P_{f(A\text{-}B)nf}$, is expected to be extremely low, in cases where agglomerates are relatively very large (*i.e.* in the case of mycelial microorganisms). To explain such a conclusion let us consider hypothetical examples:

Example 1

Let us suppose we have a mixture of equal amounts of two strains. If they are suspended in the medium as individual cells of the mean size of 1 μm^3, then for their particular cell concentration of $1.0 \cdot 10^8$ cells/mL the frequency factors could be calculated if the values of constants q_1, q_2 and q_3 could be known either by measurement or on the basis of some other method of estimation. In any case their values are expected to be dependent on particular intercellular affinity, which is supposedly much more pronounced when referring to homologous contacts, *i.e.* to contacts occurring between cells of the same strain, than when referring to heterologous contacts, *i.e.* to those occurring between cells of different strains. For a hypothetical relation

Table 1. Effect of cell and pellet sizes on the proportion of outer layer cells, n_f

Pellet diameter	n_f at v_{cell} =1 μm^3			n_f at v_{cell} =10 μm^3	
μm	s_{cell} =1 μm^2	s_{cell} =2 μm^2	s_{cell} =4 μm^2	s_{cell} =2 μm^2	s_{cell} =4 μm^2
100	6.00E-02	3.00E-02	1.50E-02	3.00E-01	1.50E-01
500	1.20E-02	6.00E-03	3.00E-03	6.00E-02	3.00E-02
1000	6.00E-03	3.00E-03	1.50E-03	3.00E-02	1.50E-02
2000	3.00E-03	1.50E-03	7.50E-04	1.50E-02	7.50E-03

$$q_1 = q_2 = q_3 = q \qquad /18/$$

and equal cell concentrations of given strains the values of frequency factors would relate similarly, *i.e.* it could be expected that

$$f_{(A\text{-}A)} = f_{(B\text{-}B)} = f_{(A\text{-}B)} = f \qquad /19/.$$

Then, the normalised values of probability coefficients would be equal as well, *i.e.* resulting in

$$P_f/q = (1.0\cdot10^8)^2 \;/\; ((1.0\cdot10^8)^2 + (1.0\cdot10^8)^2 + (1.0\cdot10^8)^2) = 1/3 \qquad /20/.$$

If for the heterologous contacts the repulsiveness would be pronounced rather than attractiveness, the value for q_3 lower than either q_1 or q_2 could be expected. For a hypothetical relation

$$q = q_1 = q_2 = 10\;q_3 \qquad /21/$$

the normalised value of probability coefficient for heterologous contacts would be

$$P_{f(A\text{-}B)} \;/\; q = 0.1\;(1.0\cdot10^8)^2 \;/\; (0.1(1.0\cdot10^8)^2 + (1.0\cdot10^8)^2 + (1.0\cdot10^8)^2) = 1/21 \qquad /22/.$$

i.e. seven times lower than in the case of equal affinities for both homologous and heterologous contacts.

Example 2

Let us suppose we have the mixed culture of equal amounts of two different strains forming agglomerates of approximately the same size and density of packing. If each cell unit in the agglomerate occupies 10 μm^3 of space and each of those belonging to agglomerate outer layer occupies 4 μm^2 of outer surface area, then for the average agglomerate diameter of 1 mm it follows that

$$V_{agl} = \pi/6\cdot d_{ag}^{\;3} = \pi/6\cdot10^9\ \mu m^3 \qquad /23/$$

$$S_{ola} = \pi\cdot d_{ag}^{\;2} = \pi\cdot10^6\ \mu m^2 \qquad /24/.$$

The total number of cells in each agglomerate would be

$$n_{clag} = \pi/6\cdot10^9 \;/\; 10 = \pi/6\cdot10^8 \qquad /25/$$

and, consequently, the number of cells belonging to agglomerate outer layer would be

$$n_{clol} = \pi/4\cdot10^6 \qquad /26/.$$

Therefore, it follows for the proportion of outer layer cells in agglomerate that

$$n_f = \pi/4\cdot10^6 \;/\; (\pi/6\cdot10^8) = 1.5\cdot10^{-2} \qquad /27/.$$

Since the homologous cell contacts exist continuously in cell agglomerates, there is no homologous contact limitation due to cell agglomeration processes. The agglomeration, one can say so, led to the increased frequency of homologous contacts. In the simplest case the values of weighting factors could be supposed to be 1.0, whereas the frequency of homologous contacts could be calculated taking into account the total number of cells, when all cells in agglomerates could be considered as viable ones, and their condition is expected to be fulfilled in the case of very soft micropellets. When the mixed microbial culture of two strains contains only 10 agglomerates of particular strain per mL, then according to equations /16/, /17/, /22/ and /27/ it follows that

$$P_{f(A\text{-}B)clag} \;/\; q = 0.1\cdot10^2\cdot(1.5\cdot10^{-2})^2 \;/\; (0.1\cdot10^2\cdot(1.5\cdot10^{-2})^2 + 2\cdot(\pi/6\cdot10\cdot10^8)^2) = 4.1\cdot10^{-21} \qquad /28/.$$

Therefore, as clearly demonstrated by equation /28/, relative probability for heterologous contacts between cells of two different strains in submerse cultures appears to be extremely low, if cells tend to form larger agglomerates, especially those of dense packing. If micropellets are formed, especially those of soft packing, the system tends to become similar to the system of suspended individual cells. Based on such a consideration one can conclude that in the case of pellet forming microorganisms the application of surface culture could lead to much more frequent heterologous contacts between microbial cells.

Example 3

Cell surface structure as well as the structure and the shape of cell agglomerates affect the frequency of cell contacts as well. This can be illustrated by analysing the properties of cell agglomerate formed as an elongated ellipsoid or cylinder of the same volume as that of the pellet elaborated in the Example 2, *i.e.* with

$$V_{agl} = \pi/6\cdot10^9\ \mu m^3 \qquad /23a/$$

for a cylinder diameter of 0.1 mm the cylinder base surface area should be

$$S_{base} = 100^2\ \pi/4 = 10^4\ \pi/4\ \mu m^2 \qquad /29/.$$

Then the cylinder height can be calculated applying the expression

$$h_{cyl} = V_{agl}/S_{base} = \pi/6\cdot10^9/(\pi/4\cdot10^4) = 2/3\cdot10^5\ \mu m \qquad /30/$$

and, consequently, for the cylinder surface area it follows that

$$S_{cyl} = 2\ S_{base} + S_{tube} = 10^4\ \pi/2 + 10^2\ \pi\cdot2/3\cdot10^5 = \text{approx. } 2\ \pi/3\cdot10^7\ \mu m^2 \qquad /31/.$$

Then the number of cells in the cylinder outer layer is determined, *i.e.*

$$n_{cylol} = 2\ \pi/3\cdot10^7/4 = \pi/6\cdot10^7 \qquad /32/$$

The proportion of outer layer cells with respect to the total number of cells in the particular agglomerate appears to be

$$n_{fcyl} = \pi/6\cdot10^7/(\pi/6\cdot10^8) = 10^{-1} \qquad /33/.$$

It is clear that such an agglomerate form is roughly 7 times more convenient for heterologous cell contacts than the spheroidal agglomerate form illustrated in Example 2. It is known that both the size and the shape of agglomerate are the functions of genetic and physiological properties of cells and cultivation conditions. As autonomous and organised systems the cells are capable of answering adequately on changes in their close environment. They can communicate with other cells. Cell signal substances (pheromones, c-AMP, AHLs, *etc.*) play dominant role in detecting the state of close environment and activities of other cells as well as in informing

the other cells. In other words, cells by their metabolism and signal substances can direct the cell movement and influence the values of kinetic constants, facilitating or aggravating the cell contacts. As shown very recently (*6,7*), the N-acylhomoserine lactones (AHLs) are used by bacteria as a barometer of cell density, in a process termed »quorum sensing«. Typically, bacteria constitutively produce and secrete small amounts of AHLs and, consequently, the AHL concentration in the culture is the function of cell density. The bacteria have transcriptional regulator proteins that become active when the AHL concentration reaches a threshold value, leading to specific target gene expression (*7*). It is generally known that, at other defined conditions, microorganism concentration, substrate and product concentrations and water activity affect the specific rates of process events (*8–10*). Therefore, it seems that in reaction systems with living cells the effects of the order and organisation are of higher relevance than in the case of other chemical and biochemical reaction systems where the effect of chaos can be more evident, at least at very low concentration of reaction components.

Relevance of contact duration

One can consider the contact necessary but not a sufficient condition for recombination to happen. The recombination between two cells can happen, if the contact between cells is enough long, *i.e.* after an adequate duration of the contact between two cells. In cell agglomerates, the cells are in a continuous contact and, therefore, there is no need for present consideration to analyse relations between cells in agglomerates. However, in the case of suspended individual cells, or very small cell-agglomerates, the duration of cell contacts should be analysed taking into account all relevant factors. The approach can be simplified as follows:

a) cells can or cannot be in the contact with other cells;

b) cells being in the contact, or being even physically linked, can become free or disconnected;

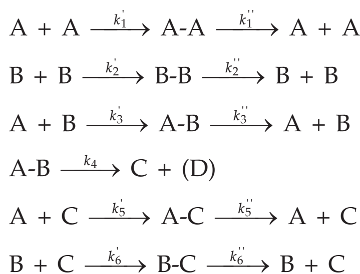
c) cells being in the contact can be capable of »producing« recombinations;

d) recombinations can result in the appearance of cells with new genetic properties;

e) cells of all strains present in the given mixed culture can be capable to grow. Any cell-recombination is a consequence of cell growth;

f) prolonged contacts increase the probability for cells to be in a simultaneous contact with many other cells.

These statements can be converted into the simplified »reaction system«:



$$A + A \xrightarrow{k_1'} A\text{-}A \xrightarrow{k_1''} A + A$$

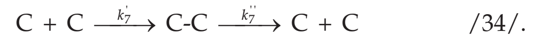
$$B + B \xrightarrow{k_2'} B\text{-}B \xrightarrow{k_2''} B + B$$

$$A + B \xrightarrow{k_3'} A\text{-}B \xrightarrow{k_3''} A + B$$

$$A\text{-}B \xrightarrow{k_4} C + (D)$$

$$A + C \xrightarrow{k_5'} A\text{-}C \xrightarrow{k_5''} A + C$$

$$B + C \xrightarrow{k_6'} B\text{-}C \xrightarrow{k_6''} B + C$$



$$C + C \xrightarrow{k_7'} C\text{-}C \xrightarrow{k_7''} C + C \qquad /34/.$$

If neglecting the appearance and the growth of another new strain (D), the kinetics of changes of the concentration of free cells and »cells in contact« can be described by the system of differential equations

$$dN_{A\text{-}A}/dt = k_1' \cdot N_A^2 - k_1'' \cdot N_{A\text{-}A} \qquad /35/$$

$$dN_{B\text{-}B}/dt = k_2' \cdot N_B^2 - k_2'' \cdot N_{B\text{-}B} \qquad /36/$$

$$dN_{A\text{-}B}/dt = k_3' \cdot N_A \cdot N_B - k_3'' \cdot N_{A\text{-}B} - k_4 \cdot N_{A\text{-}B} \qquad /37/$$

$$dN_C/dt = k_4 \cdot N_{A\text{-}B} - k_5' \cdot N_A \cdot N_C + k_5'' \cdot N_{A\text{-}C} - k_6' \cdot N_B \cdot N_C + k_6'' \cdot N_{B\text{-}C} - k_7' \cdot N_C^2 + 2k_7'' \cdot N_{C\text{-}C} + f_G(N_C) \qquad /38/$$

$$dN_A/dt = - k1' \cdot N_A^2 + 2k_1'' \cdot N_{A\text{-}A} - k_3' \cdot N_A \cdot N_B + k_3'' \cdot N_{A\text{-}B} - k_5' \cdot N_A \cdot N_C + k_5'' \cdot N_{A\text{-}C} + f_G(N_A) \qquad /39/$$

$$dN_B/dt = - k_2' \cdot N_B^2 + 2k_2'' \cdot N_{B\text{-}B} - k_3' \cdot N_A \cdot N_B + k_3'' \cdot N_{A\text{-}B} - k_6' \cdot N_B \cdot N_C + k_6'' \cdot N_{B\text{-}C} + f_G(N_B) \qquad /40/$$

$$dN_{A\text{-}C}/dt = k_5' \cdot N_A \cdot N_C - k_5'' \cdot N_{A\text{-}C} \qquad /41/$$

$$dN_{B\text{-}C}/dt = k_6' \cdot N_B \cdot N_C - k_6'' \cdot N_{B\text{-}C} \qquad /42/$$

$$dN_{C\text{-}C}/dt = k_7' \cdot N_C^2 - k_7'' \cdot N_{C\text{-}C} \qquad /43/$$

$$f_G(N_A) = \mu_A \cdot N_A \qquad /44/$$

$$f_G(N_B) = \mu_B \cdot N_B \qquad /45/$$

$$f_G(N_C) = \mu_C \cdot N_C \qquad /46/$$

where the specific growth rate, μ, can be the function of a number of factors. Various mathematical models can be applied for expressing μ values *(3,10)*, and in the case of cell agglomerate formation the three-dimensional growth concept should be taken into consideration when applying mathematical models *(3,8,10)*. In any moment the total number of »bio-particles« is

$$N_{bio} = N_A + N_B + N_C + N_{A\text{-}A} + N_{B\text{-}B} + N_{C\text{-}C} + N_{A\text{-}B} + N_{A\text{-}C} + N_{B\text{-}C} \qquad /47/.$$

To see whether the system of differential equations /35–47/ describes the »reaction system« adequately, a computer simulation was performed applying the hypothetical parameter values shown in Table 2.

Simulation results are presented in Fig. 4 and Table 3. Data suggest that the applied mathematical model can roughly well explain kinetics of main process events in the supposed »reaction system«, if a short process period is considered. However, we should point out that although the process considered in this work appears to be rather stochastic than deterministic, the deterministic mathematical model was applied to explain the process events. Presented computer simulation data suggest that such an approach could be accepted as the first step prior to performing a series of appropriate experiments, which should be the basis for the second step, *i.e.* for the more complete and more precise description of relevant real process events. In any case, the obtained data encourage us to extend this mathematical model further, in order to explain the kinetics of process events better.

Table 2a. Parameter values

Parameter	Value	
$k1'$	$1\cdot10^{-9}$	/ mL h^{-1}
$k1''$	$1\cdot10^{-8}$	/ h^{-1}
$k2'$	$1\cdot10^{-9}$	/ mL h^{-1}
$k2''$	$1\cdot10^{-8}$	/ h^{-1}
$k3'$	$1\cdot10^{-10}$	/ mL h^{-1}
$k3''$	$2\cdot10^{-10}$	/ h^{-1}
$k4$	$1\cdot10^{-1}$	/ h^{-1}
$k5'$	$1\cdot10^{-10}$	/ mL h^{-1}
$k5''$	$1\cdot10^{-9}$	/ h^{-1}
$k6'$	$5\cdot10^{-10}$	/ mL h^{-1}
$k6''$	$1\cdot10^{-9}$	/ h^{-1}
$k7'$	$1\cdot10^{-9}$	/ mL h^{-1}
$k7''$	$2\cdot10^{-10}$	/ h^{-1}
μ_A	$5\cdot10^{-2}$	/ h^{-1}
μ_B	$6\cdot10^{-2}$	/ h^{-1}
μ_C	$8\cdot10^{-2}$	/ h^{-1}

Table 2b. Initial conditions

Parameter	Value	
t	0	/ h
N_A	$1\cdot10^5$	/ mL^{-1}
N_B	$1\cdot10^5$	/ mL^{-1}
N_C	0	/ mL^{-1}
N_{aa}	0	/ mL^{-1}
N_{bb}	0	/ mL^{-1}
N_{cc}	0	/ mL^{-1}
N_{ab}	0	/ mL^{-1}
N_{bc}	0	/ mL^{-1}

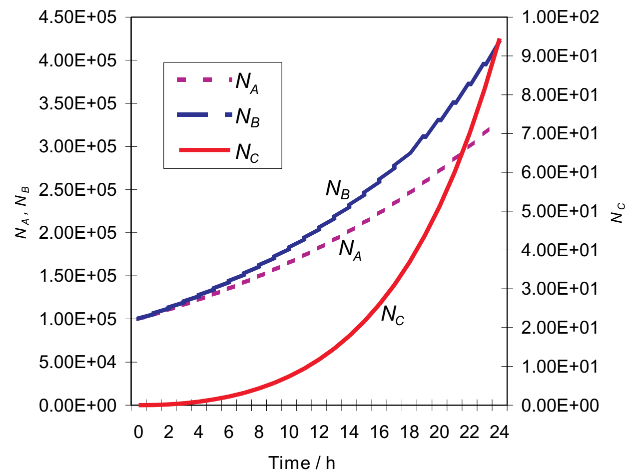


Fig. 4. Growth kinetics of parent and child strains

Table 3. Changes of particle numbers during the process

Time	N_A	N_B	N_C	N_{aa}	N_{bb}	N_{ab}	N_{ac}	N_{cc}	N_{bc}	N_{bio}
h	mL^{-1}	mL^{-1}	mL^{-1}	mL^{-1}	mL^{-1}	mL^{-1}	mL^{-1}	mL^{-1}	mL^{-1}	mL^{-1}
0	100000	100000	0	0	0	0	0	0	0	200000
4	122081	127062	0.91589	49.1577	51.313	4.19957	0.00014	6.4E-10	0.00071	249248.1
8	149021	161426	4.32303	122.413	134.145	9.32847	0.00144	2.7E-08	0.00768	310716.6
12	181881	205049	11.7832	231.55	267.816	16.3524	0.00655	2.8E-07	0.03603	387457.6
16	221950	260406	25.9513	394.098	483.448	26.6155	0.02136	1.7E-06	0.12145	483286.9
20	270793	330619	51.1864	636.106	831.127	42.0946	0.05851	7.5E-06	0.34443	602972.9
24	330301	419621	94.5052	996.253	1391.370	65.7744	0.14439	2.8E-05	0.88065	752471.6

In microbial cultures, simultaneously with the growth of microorganisms, the uptake of substrates and the formation of products take place. Different methods of microorganism cultivation can be applied. In the batch cultures (BC) the rates of process events and the concentrations of »reaction components« change continuously, whereas in the continuous cultures (CC) the constant culture properties with relatively well defined small oscillations can be maintained, if the cultivation conditions are determined to discriminate the appearance of any »culture degeneration«. The specific rates of process events in microbial cultures depend on properties of microbial cells as well as on cultivation conditions, *i.e.* on the close environment of microbial cells, mainly determined by concentrations of different substances (*e.g.* different substrates, metabolites, enzymes, inhibitors, *etc.*) and the mass transfer phenomena during the process. Although different mathematical models can be applied to describe kinetics of process events in microbial cultures, in our present consideration we decided to apply the mathematical model based on known approaches for describing particular process events. Since in the real processes developed on the basis of the use of complex substrates, the information on growth limiting factors can be often inadequate, therefore we decided to base our approach on the application of modified logistic equations instead of equations of Michaelis-Menten-Monod type. We focused on the development of the model applicable for hypothetical BC and CC. Naturally, in developing such a model an extension of equations /35–47/ showed to be necessary. Therefore, the following mathematical model was applied:

Growth kinetics

$$dN_{A\text{-}A}/dt = k_1'\cdot N_A^2 - k_1''\cdot N_{A\text{-}A} - \delta\cdot N_{A\text{-}A} \qquad /48/$$

$$dN_{B\text{-}B}/dt = k_2'\cdot N_B^2 - k_2''\cdot N_{B\text{-}B} - \delta\cdot N_{B\text{-}B} \qquad /49/$$

$$dN_{A\text{-}B}/dt = k_3'\cdot N_A\cdot N_B - k_3''\cdot N_{A\text{-}B} - k_4\cdot N_{A\text{-}B} - \delta\cdot N_{A\text{-}B} \qquad /50/$$

$$dN_{C\text{-}C}/dt = k_7'\cdot N_C^2 - k_7''\cdot N_{C\text{-}C} - \delta\cdot N_{C\text{-}C} \qquad /51/$$

$$dN_{A\text{-}C}/dt = k_5'\cdot N_A\cdot N_C - k_5''\cdot N_{A\text{-}C} - \delta\cdot N_{A\text{-}C} \qquad /52/$$

$$dN_{B\text{-}C}/dt = k_6'\cdot N_B\cdot N_C - k_6''\cdot N_{B\text{-}C} - \delta\cdot N_{B\text{-}C} \qquad /53/$$

$$dN_A/dt = -\,k_1'\cdot N_A^2 + 2\cdot k_1''\cdot N_{A\text{-}A} - k_3'\cdot N_A\cdot N_B + k_3''\cdot N_{A\text{-}B} - k_5'\cdot N_A\cdot N_C + k_5''\cdot N_{A\text{-}C} + \mu_A\cdot N_A\cdot(1 - N_{bio}/N_{bimax}) - \delta\cdot N_A \qquad /54/$$

$$dN_B/dt = -\,k_2'\cdot N_B^2 + 2\cdot k_2''\cdot N_{B\text{-}B} - k_3'\cdot N_A\cdot N_B + k_3''\cdot N_{A\text{-}B} - k_6'\cdot N_B\cdot N_C + k_6''\cdot N_{B\text{-}C} + \mu_B\cdot N_B\cdot(1 - N_{bio}/N_{bimax}) - \delta\cdot N_B \qquad /55/$$

Table 4. Initial conditions and parameters

Parameter	Value		Parameter	Value	
$k1'$	$2\cdot10^{-13}$	/ mL h^{-1}	N_A	$1\cdot10^{5}$	/ mL^{-1}
$k1'$	$1\cdot10^{-7}$	/ h^{-1}	N_B	$1\cdot10^{5}$	/ mL^{-1}
$k2'$	$2\cdot10^{-13}$	/ mL h^{-1}	N_C	0	/ mL^{-1}
$k2''$	$1\cdot10^{-7}$	/ h^{-1}	N_D	0	/ mL^{-1}
$k3'$	$2\cdot10^{-13}$	/ mL h^{-1}	N_{aa}	0	/ mL^{-1}
$k3''$	$2\cdot10^{-7}$	/ h^{-1}	N_{bb}	0	/ mL^{-1}
$k4$	$1\cdot10^{-3}$	/ h^{-1}	N_{cc}	0	/ mL^{-1}
$k5'$	$1\cdot10^{-13}$	/ mL h^{-1}	N_{ab}	0	/ mL^{-1}
$k5''$	$2\cdot10^{-7}$	/ h^{-1}	N_{bc}	0	/ mL^{-1}
$k6'$	$2\cdot10^{-13}$	/ mL h^{-1}	N_{cc}	0	/ mL^{-1}
$k6''$	$1\cdot10^{-7}$	/ h^{-1}	N_{bio}	$2\cdot10^{5}$	/ mL^{-1}
$k7'$	$2\cdot10^{-13}$	/ mL h^{-1}	N_{bimax}	$1\cdot10^{9}$	/ mL^{-1}
$k7''$	$1\cdot10^{-7}$	/ h^{-1}	γ_s	120	/ mg mL^{-1}
μ_A	0.2	/ h^{-1}	γ_{LO2}	0.001	/ mg mL^{-1}
μ_B	0.15	/ h^{-1}	γ^*_{LO2}	$7\cdot10^{-3}$	/ mg mL^{-1}
μ_C	0.25	/ h^{-1}	$\gamma_{LO2\ s}$	$6\cdot10^{-3}$	/ mg mL^{-1}
μ_D	0	/ h^{-1}	γ_{pA}	0	/ mg mL^{-1}
$\delta_{(CC)}$	0.15; 0.075	/ h^{-1}	γ_{pB}	0	/ mg mL^{-1}
$\delta_{(BC)}$	0	/ h^{-1}	γ_{pC}	0	/ mg mL^{-1}
q_{ms}	$3\cdot10^{-9}$	/ mg h^{-1}	q_{sC}	$2\cdot10^{-10}$	/ mg h^{-1}
q_{ox}	$1\cdot10^{-9}$	/ mg h^{-1}	k_{sO2}	$7\cdot10^{-5}$	/ mg mL^{-1}
q_{pA}	$3\cdot10^{-9}$	/ mg h^{-1}	k_{ss}	5	/ mg mL^{-1}
q_{pB}	$3\cdot10^{-9}$	/ mg h^{-1}	k_{spA}	0.5	/ mg mL^{-1}
q_{pC}	$1\cdot10^{-10}$	/ mg h^{-1}	k_{spB}	0.5	/ mg mL^{-1}
q_{sB}	$1.2\cdot10^{-10}$	/ mg h^{-1}	k_{pC}	0.01	/ h^{-1}
t	0	/ h	k_{La}	150	/ h^{-1}

$$dN_C/dt = k_4\cdot N_{A\text{-}B} - k_5'\cdot N_A\cdot N_C + k_5''\cdot N_{A\text{-}C} - k_6'\cdot N_B\cdot N_C + k_6''\cdot N_{B\text{-}C} - k_7'\cdot N_C^{\,2} + 2\cdot k_7''\cdot N_{C\text{-}C} + \mu_C\cdot N_C\cdot(1 - N_{bio}/N_{bimax}) - \delta\cdot N_C \qquad /56/$$

$$dN_D/dt = k_4\cdot N_{A\text{-}B} + \mu_D\cdot(1 - N_{bio}/N_{bimax}) - \delta\cdot N_D \qquad /57/$$

supposing the case where the growth of the strain D and, consequently, the contacts of its cells could be neglected (μ_D=0). Otherwise an approach analogous to that for the strain C should be applied

$$dN_{bio}/dt = dN_A/dt + dN_B/dt + dN_C/dt + dN_D/dt + dN_{A\text{-}A}/dt + dN_{B\text{-}B}/dt + dN_{C\text{-}C}/dt + dN_{A\text{-}B}/dt + dN_{A\text{-}C}/dt + dN_{B\text{-}C}/dt \qquad /58/.$$

Substrate uptake kinetics

Since the present description of growth kinetics is based on the application of modified logistic equation the kinetics of the consumption of growth limiting substrates can be neglected in the primary steps of mathematical model development. Therefore, our interest can be focused on the mathematical description of the kinetics of the substrate relevant for a product formation and the culture maintenance. Then, the following equation

$$d\gamma_s/dt = -q_{ms}\cdot(\gamma_s/(k_{ss} + \gamma_s)\cdot N_{bio} + \delta\cdot(\gamma_{s0} - \gamma_s) \qquad /59/$$

can be recommended to be applied (it is supposed that all bioparticles consume the substrate). In aerobic cultures the availability of oxygen is necessary and the oxygen should be supplied continuously due to its very low solubility in water solutions. Commonly, oxygen transfer and oxygen uptake rates can roughly be expressed applying the equation

$$d\gamma_{LO2}/dt = k_La\cdot(\gamma^*_{LO2} - \gamma_{LO2}) - q_{ox}\cdot(\gamma_{LO2}/(k_{sO2} + \gamma_{LO2}))\cdot N_{bio} + \delta\cdot(\gamma_{LO2s} - \gamma_{LO2}) \qquad /60/.$$

Product formation kinetics

In mixed microbial cultures of three different strains the formation of different specific products can be supposed. One of possibilities is that the strain A produces the product *PA* which is then used by the strain B to convert it into the product *PB*. This is converted into the product *PC* after being consumed by the new strain C. Some instability of formed product *PC* can also be supposed. Such a hypothesis for product formation kinetics suggests the application of mathematical equations as follows:

$$d\gamma_{pA}/dt = q_{pA}\cdot(\gamma_s/(k_{ss} + \gamma_s))\cdot N_A - q_{sB}\cdot N_B\cdot(\gamma_{pA}/(k_{spA} + \gamma_{pA})) - \delta\cdot\gamma_{pA} \qquad /61/$$

$$d\gamma_{pB}/dt = q_{pB}\cdot N_B\cdot(\gamma_{pA}/(k_{spA} + \gamma_{pA})) - q_{sc}\cdot N_C\cdot(\gamma_{pB}/(k_{spB} + \gamma_{pB})) - \delta\cdot\gamma_{pB} \qquad /62/$$

$$d\gamma_{pC}/dt = q_{pC}\cdot N_C\cdot(\gamma_{pB}/(k_{spB} + \gamma_{pB})) - k_{pC}\cdot\gamma_{pC} - \delta\cdot\gamma_{pC} \qquad /63/$$

Mathematical model represented by equations /48–63/ is applicable for both BC and CC. As in the case of simpler mathematical application (Fig. 4 and Table 3) the computer simulation was also applied for solving the system of equations /48–63/. To perform simulations, the hypothetical parameter values mainly close to those which can be observed in the real microbial cultures and the initial conditions indicated in Table 4, were applied. Results are demonstrated in Figs. 5 and 6, and Table 5. Data clearly show that the applied mathematical model can well explain the process relationships as well as the kinetics of particular process events in both BC (δ = 0.0) and CC. As demonstrated, the survival and the growth rate of new strain largely depend on its viability with respect to applied culture conditions, especially when taking into account viabilities of other culture members. Quite as expected for the strains differing in specific growth rates, CC appears to be much more favourable for the superior than for the inferior strains. Predomination of superior strains (A,C) and a »wash out« of inferior strains (B,D) are normal consequences. However, the rate of new product formation can essentially depend on microbial population structure, *i.e.* on the establishing of some kind of microbial consortium. Such a consortium can be a prerequisite for an efficient new product formation (Table 5). Very pronounced differences in specific growth rates were chosen for simulations in order to elucidate better the consequences of the application of different cultivation conditions.

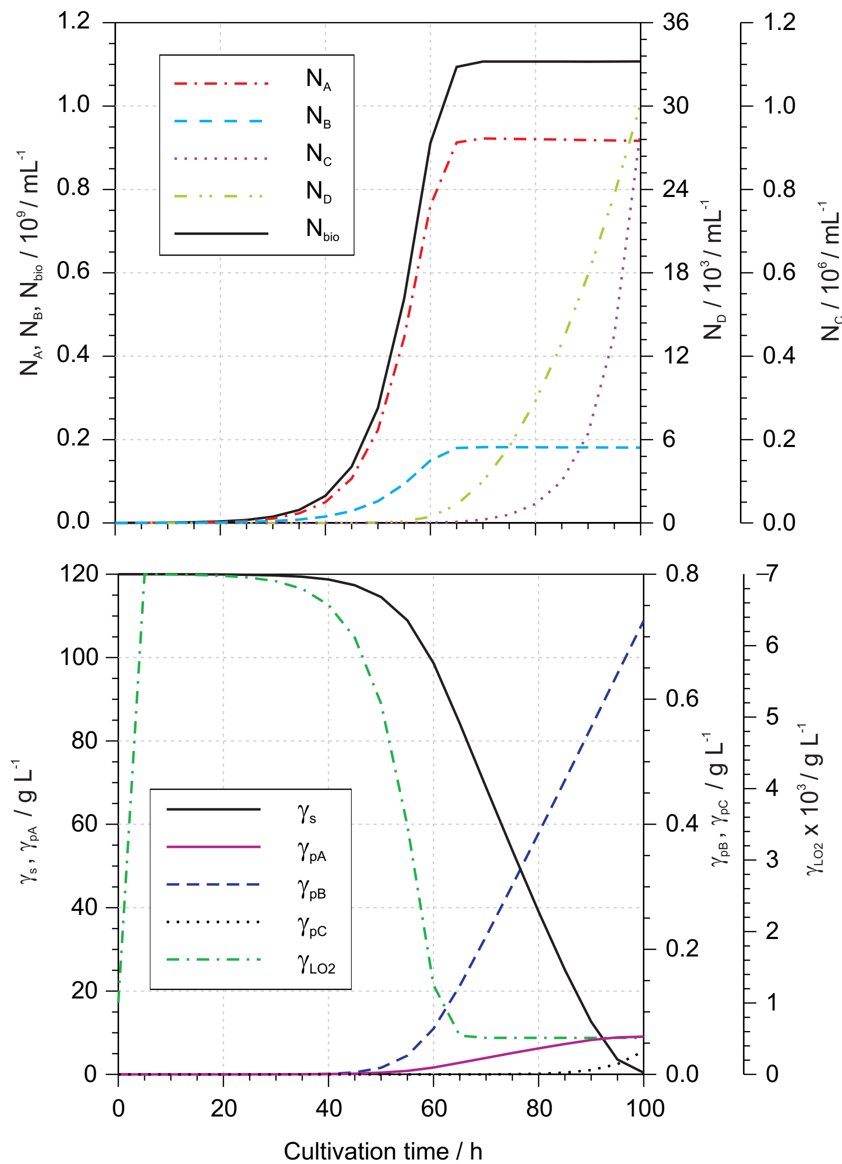


Fig. 5. Batch process course of mixed microbial culture

Table 5. Computer simulation data: Effect of cultivation conditions on mixed microbial population structure and change of concentration of substrates and products

Time	N_A	N_B	N_C	N_D	N_{AA}	N_{BB}	N_{CC}	N_{AB}	N_{AC}	N_{BC}	N_{bio}	γ_S	γ_{pA}	γ_{pB}	γ_{pC}	γ_{LO2}
h	mL^{-1}											mg mL^{-1}				
0	$1\cdot10^5$	$1\cdot10^5$	0	0	0	0	0	0	0	0	$2\cdot10^5$	120	0	0	0	0.001
100 "BC"	$8.99\cdot10^8$	$9.25\cdot10^7$	$1.10\cdot10^4$	$1.06\cdot10^4$	$8.01\cdot10^6$	$8.78\cdot10^4$	$2.64\cdot10^{-4}$	$4.08\cdot10^5$	18.25	3.76	$1.0\cdot10^9$	$2.08\cdot10^{-2}$	10.25	0.45	$7.0\cdot10^{-6}$	$8.44\cdot10^{-4}$
300 "CC"	$2.46\cdot10^8$	$1.94\cdot10^4$	$5.62\cdot10^6$	$3.78\cdot10^{-2}$	$8.14\cdot10^4$	$1.01\cdot10^{-3}$	28.22	4.24	742.3	0.146	$2.52\cdot10^8$	115.18	0.47	$8.30\cdot10^{-6}$	$5.88\cdot10^{-8}$	$5.34\cdot10^{-3}$
400 "BC"	$9.52\cdot10^8$	$5.38\cdot10^4$	$3.09\cdot10^7$	21.15	$1.66\cdot10^7$	$5.41\cdot10^{-2}$	$1.70\cdot10^4$	451.2	$2.65\cdot10^5$	30.11	$1.0\cdot10^9$	$6.21\cdot10^{-13}$	11.6	$2.85\cdot10^{-4}$	$7.12\cdot10^{-5}$	$8.44\cdot10^{-4}$
400 "BC"*	$9.52\cdot10^8$	$5.38\cdot10^4$	$3.09\cdot10^7$	21.15	$1.66\cdot10^7$	$5.41\cdot10^{-2}$	$1.70\cdot10^4$	451.2	$2.65\cdot10^5$	30.11	$1.0\cdot10^9$	$6.21\cdot10^{-13}$	11.6	$2.98\cdot10^{-2}$ *	$1.63\cdot10^{-2}$*	
300 "CC"**	$6.21\cdot10^8$	$1.66\cdot10^6$	$5.25\cdot10^5$	31.76	$1.03\cdot10^6$	14.59	0.49	$1.8\cdot10^3$	346.8	2.31	$6.24\cdot10^8$	96.27	2.36	$2.42\cdot10^{-3}$	$2.96\cdot10^{-6}$	$2.94\cdot10^{-3}$

BC = batch cultivation period
CC = continuous cultivation period (dilution rate δ = 0.15 h^{-1})
* = at BC start (end of CC = 300th hour) the value γ_{pB} = $8.30\cdot10^{-2}$ was applied
** = dilution rate δ = 0.075 h^{-1} was applied

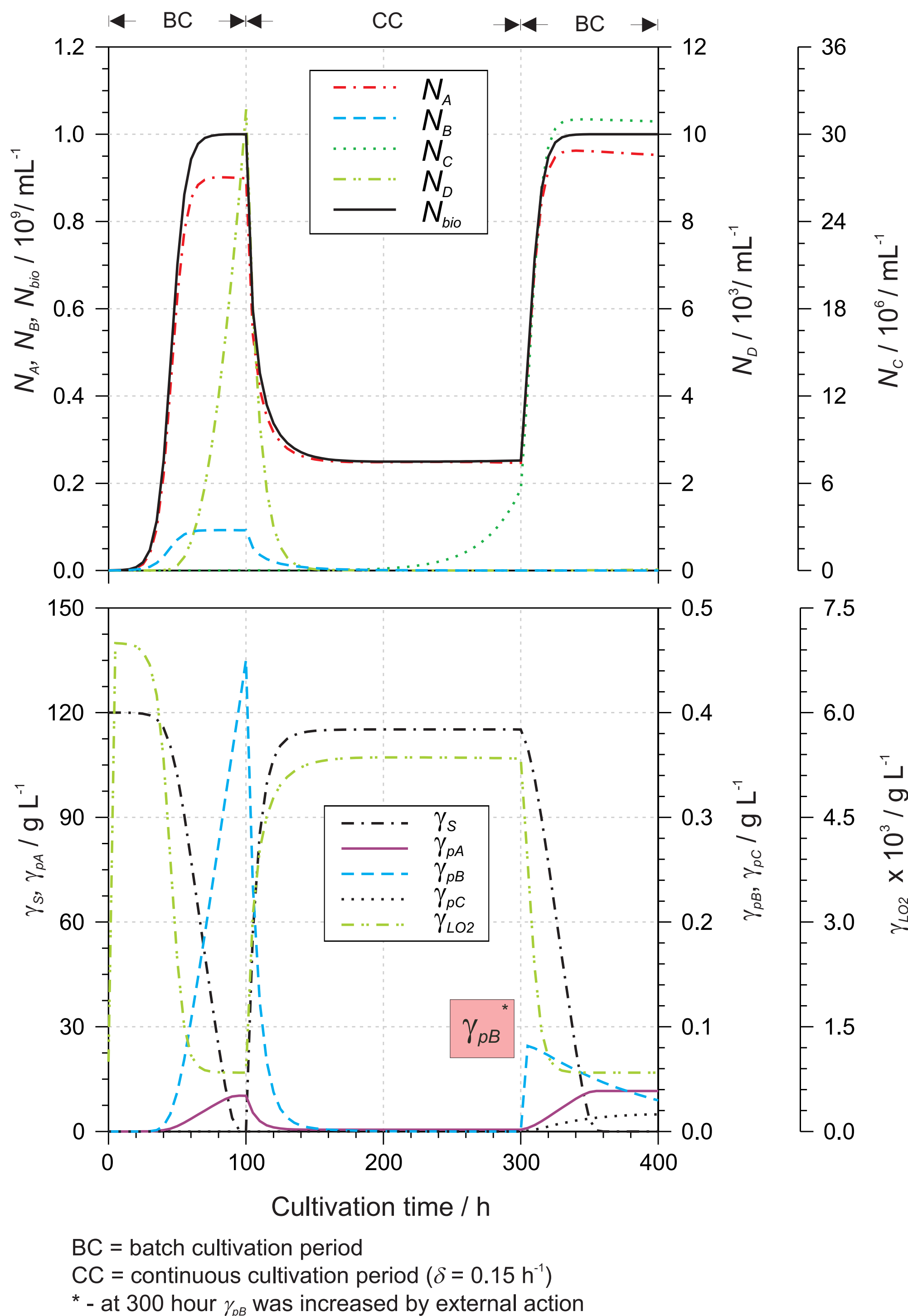


Fig. 6. Effects of application of different cultivation methods on process course of mixed microbial culture

Now, the question arises how to recognise bioparticles and how to determine their specific participation. Potentials of direct insight techniques are very limited. Fortunately, already developed methods for the detection of specific cell activities and formed specific metabolites are a good basis for developing specific reliable methods for the identification and separation of particles appearing in the focus of our main interest. Continuous progress is evident. One of the recent information refers to the improvement of microorganism screening. The efficiency of both microorganism screening and bioactive substance detection can be significantly improved by applying agarised media layers fortified with inserted metallic nets *(11,12)*. Preparing or adapting of particles to be suitable for prolonged contacts and maintaining culture conditions favourable for heterologous recombinations are important factors in every procedure for obtaining microbial cells with created new genetic properties. In this consideration, the effect of a series of other factors is neglected because of the need to give a

rough insight into the main relevant events. Thus, in this consideration the genetic history of a particular strain and its effect on overall results of planned recombinations were not analysed.

Surface Cultures

When cultivated on solid, *i.e.* agarised media, the microorganisms grow on media surfaces and express their activities, which can be protective or inhibitory ones. The surface occupied by microbial cells increases during the growth, and contacts between different colonies can result. If colonies of different strains are cultivated, the heterologous cell contacts become possible. Theoretically, the frequency of contacts can be directed by experiment design. If during the growth the cells of one microbial strain do not produce substances inhibiting the growth of other strain, the experiment can be designed in such a way that enables heterologous contacts with high probability.

Let us consider that the square plate of agarised medium layer of the surface area is $10 \cdot 10 = 100$ cm^2. If the experiment is designed that the plate contains 100 equidistant lines of microbial cells of two different strains »A« and »B«, (100–2) = 98 lines of heterologous contact is possible. The length of every contact line is $100 \cdot 1000$ μm = 10^5 μm, *i.e.* the equivalent for 10^5 cells of one strain which could be in the contact with the same number of cells of the other strain, *i.e.* the equivalent which refers to 10^5 of heterologous cell contacts/contact line. Therefore, $98 \cdot 10^5$ = approx. 10^7 of heterologous contacts is possible. If the height of contact lines is 0.1 mm = 10^2 μm, then the total number of heterologous contacts could be 10^9. The total number of cells (of 1 μm^3 size) could be $(10 \cdot 10 \cdot 10^3)^2 \cdot 10^2 = 10^{12}$ / plate. It follows for contact yield that

$$n_{hclc} = 10^9/10^{12} = 10^{-3} \qquad /64/.$$

If microbial lines would be crossed (rectangularly), the contact yield could be expressed as

$$n_{hclc} \geq 2 \cdot 10^{-3} \qquad /65/,$$

because in 50 % of the total surface area the well mixed population of two strains would be present. Of course, there is none guaranty for heterologous contacts to be fruitful with respect to recombinations between microbial cells. The possibility of heterologous contacts could also be significantly reduced or even eliminated, if during the growth the production of substances acting inhibitory on cell growth of any of cultivated microorganisms would be initiated.

Microbial Biomass Kneading

One of the possibilities for increasing the frequency of heterologous contacts between microbial cells could be the kneading of concentrated microbial suspensions or separated wet biomass of different strains. Culture conditions enabling the growth of microbial cells in the mixture is one of prerequisites for contacts to be fruitful. In the case of too high concentration of aerobic viable microbial cells the oxygen supply should be satisfactory, if the cell viability is maintained. Technically, it is not easy to maintain satisfactory oxygen supply in such cultures because of too high oxygen uptake rates. However, the exit can be found. First of all, a biomass kneading instead of culture stirring should be applied, *i.e.* the cultivation should be performed in bioreactors of special design. The principles of slow cell movement and continuous exposition of cells to oxygen should be strongly taken into account when designing bioreactors for a biomass kneading of different strains. Systems based on the passing of wet air over surfaces of thin culture layers seems to be one of the possible solutions. Bioreactor can be designed in such a way that layers can be repeatedly renewed and fed with nutrients.

Specific Approaches

To increase the probability of genetic modifications very efficient specific methods were developed. These are based on specific approaches where limitations by factors of the mentioned group 4) are minimised. As documented and pointed out in some reports *(13,14)*, methods based on the fusion of protoplasts *(13)* and those based on the application of electroduction *(14)* proved to be very efficient in transferring genetic materials from one cell to the others. In the case of electroduction, suspension of microbial donor and recipient cells is exposed to a short-duration, high-amplitude electrical pulse to cause reversible permeabilization of the cell membrane. Transfer of DNA molecules can occur both intraspecifically and/or interspecifically. Direct plasmid transfer between two closely and/or distantly related bacterial species can be achieved *(14)*. The aim of this work was not to analyse specific methods in details, since it seems more appropriate to treat them when focusing to factors of mentioned classes 1) to 3).

Remarks

Biological nature of bioparticles

The fact that microorganisms belong to live organisms, should always be taken into account. In general, the ability of microorganisms to adapt their cells in accordance with changes of their environment, their reproduction ways and their behaviour in their cultures are important factors. Microorganisms are capable to communicate, some of them even move, but their movement can be produced also by an external force, frequently causing an adequate cell response. External force can induce or even lead to cell contacts, but it can also damage or even destroy the cells. As actually known, dead cells in their contacts do not produce live cells, although their presence and presence of products of their autolysis in culture affect live cells. In their cultures, microorganisms by their growth and metabolism affect the properties of their environment and, *vice versa*, changes in microbial environment reflect on properties of microbial cells. One of the goals of *metabolic engineering* or *cell engineering* is to develop holistic metabolic models of living cells, *i.e.* such models where cell is considered as a complex network of material and energy balances, kinetics of enzyme reactions, and sophisticated

hierarchy of regulatory blocks and circuits *(15,16)*. Co-ordination of whole blocks or networks of metabolic pathways is required for complex microbial activities *(16,17)*. Culture history and, therefore a history of particular cells, influence cell properties and cell behaviour in any moment of cell life. Therefore, since cells that have been in the contacts can differ »historically« from those having not been in the contact (if the contacts have been enough long and »memorised«), the equation /39/, *e.g.* in the moment of process start, could be converted into the equations

$$dN_{AT}/dt = dN_A/dt + dN_{A1}/dt + dN_{A2}/dt + dN_{A3}/dt + f_G(N_{AT}) \quad /39a/$$

$$dN_A/dt = -k_1'\cdot N_A^2 - k_3'\cdot N_A\cdot N_B - k_5'\cdot N_A\cdot N_C \quad /39b/$$

$$dN_{A1}/dt = 2k_1''\cdot N_{A\text{-}A} \quad /39c/$$

$$dN_{A2}/dt = k_3''\cdot N_{A\text{-}B} \quad /39d/$$

$$dN_{A3}/dt = k_5''\cdot N_{A\text{-}C} \quad /39e/$$

$$f_G(N_{AT}) = \mu_{AT}\cdot N_{AT} \quad /39f/$$

where N_{AT} means the total number of cells of the strain »A«. If the contacts become forgotten then

$$dN_{A1}/dt = 2\,k_1''.N_{A\text{-}A} - k_{f1}\cdot N_{A1} \quad /39cf/$$

$$dN_{A2}/dt = k_3''\cdot N_{A\text{-}B} - k_{f2}\cdot N_{A2} \quad /39df/$$

$$dN_{A3}/dt = k_5''\cdot N_{A\text{-}C} - k_{f3}\cdot N_{A3} \quad /39ef/$$

$$dN_A/dt = -k_1'\cdot N_A^2 - k_3'\cdot N_A\cdot N_B - k_5'\cdot N_A\cdot N_C + k_{f1}\cdot N_{A1} + k_{f2}\cdot N_{A2} + k_{f3}\cdot N_{A3} \quad /39bf/.$$

The analysis could be extended further taking into account numbers of different homologous and heterologous contacts, *etc*. In their contacts cells can transfer their information and material particles to other cells. The cell enriched with new information or even with some cell parts (*e.g.* with plasmids) differs from that having not such an information or even having lost something own.

Microbial process energy

In the presented mathematical model the energy changes in the hypothetical mixed microbial culture have not been considered, because of the intention to elaborate them specifically, during the further phases of model development, taking into account the fact that microbial cell itself is a complex system, a bioreactor where different simple or complex chemical compounds, in a series of catabolic reactions (degradation, oxidation), are transformed into simple compounds (with simultaneous energy evolution) which during the further series of anabolic reactions (reduction, biosynthesis) are converted into microbial biomass and a series of chemical compounds of different molecular mass as bioprocess products. One can expect that a simple approach to the description of kinetics of energy changes can lead to the appropriate mathematical model, as proven experimentally in describing kinetics of heat evolution during the process of oxytetracycline biosynthesis in industrial bioreactors (*18*).

Computer simulation

Based on the mathematical model the corresponding simulation programme suitable for the *MicroMath Scientist Ver 2.0* software package was developed and a series of computer simulations was performed applaying PC compatible computer. The integration methods was Error Controlled Runge-Kutta with relative error set to $1\cdot10^{-6}$, and the maximum number of steps was set to 5000. Selected examples of simulation processes were choosen to be demonstrated in this work.

Ethics

For a question whether to put in contacts the cells differing in their genetic properties, if it is expected that they could produce new cells with properties dangerous for humans and the environment in general, the answer should be certainly not, except (if at all) when all circumstances for a safe control of possible risks are fulfilled.

Symbols

A, B, C, D,... – denotations of reaction components or reagents, or bioparticles

A-A, A-B, A-C, B-B, B-C, C-C,... – denotations of the kind of bio-particle contacts

C_A, C_B, C_C, C_D,.. – reagent concentrations, $M\ L^{-3}$

d – variable derivative denotation

d_{ag} – agglomerate diameter, L

dN_A/dt, $dN_{A\text{-}A}/dt$, $dN_{A\text{-}B}/dt$, $dN_{A\text{-}C}/dt$, dN_B/dt, $dN_{B\text{-}B}/dt$, $dN_{B\text{-}C}/dt$, dN_C/dt, $dN_{C\text{-}C}/dt$, dN_D/dt, *etc.* – derivative of N_A, $N_{A\text{-}A}$, *etc.*, with respect to t; rates of changes of denoted cell number or bio-particle number concentrations, $L^{-3}\ T^{-1}$

$f_{A\text{-}A}$, $f_{B\text{-}B}$, $f_{A\text{-}B}$,... – factors of relative frequency of denoted contacts between bio-particles, $L^{-3}\ T^{-1}$

$f_{(A\text{-}B)nf}$ – factor of relative frequency of heterogeneous contacts referring to agglomerates of the strain A and the strain B, $L^{-3}\ T^{-1}$

$f_{G(NA)}$, $f_{G(NB)}$, $f_{G(NC)}$,... – growth rates of denoted microbial strain cell numbers, $L^{-3}\ T^{-1}$

h_{cyl} – height of cylindrical agglomerate, L

k_1, k_2,... – reaction rate constants; dimensions:
a) first order kinetics, T^{-1}
b) 2nd order kinetics, $M^{-1}\ L^3\ T^{-1}$

K – equilibrium constant referring to reversible reaction system, *dimensionless*

k_1', k_2', k_3',... – rate constants referring to cell contacts, $L^3\ T^{-1}$

k_1'', k_2'', k_3'',... – rate constants referring to the dissociation of formed cell contacts, T^{-1}

k_{f1}, k_{f2}, k_{f3},... – rate constants of cell contact forgetting, T^{-1}

k_La – volumetric coefficient of oxygen transfer rate, T^{-1}

k_{pC} – rate constant of product PC degradation, T^{-1}

k_{sO_2} – saturation constant referring to oxygen, $M\ L^{-3}$

k_{spA} – saturation constant referring to the product PA, $M\ L^{-3}$

k_{spB} – saturation constant referring to the product PB, $M\ L^{-3}$

k_{ss} – saturation constant referring to the substrate, $M\ L^{-3}$

L – length

M – mass

M^* – mass, when it refers to specific rates of product formation and substrate uptake with reference to one microbial cell

N_A, N_B, N_C, N_D, $N_{A\text{-}A}$, $N_{A\text{-}B}$, $N_{A\text{-}C}$, $N_{B\text{-}B}$, $N_{B\text{-}C}$, $N_{C\text{-}C}$,... – numbers of denoted cells or bio-particles in the given volume, L^{-3}

N_{bimax} – theoretically maximal number of bioparticles in the given volume, L^{-3}

N_{bio} – number of bioparticles in the given volume, L^{-3}

n_{clag} – total number of cells in particular agglomerate, *dimensionless*

n_{clol} – total number of cells in agglomerate outer layer, *dimensionless*

n_f – cell fraction, *i.e.* proportion of outer layer cells with respect the total number of cells in agglomerate, *dimensionless*

n_{fcyl} – fraction of outer layer cells with respect to the total number of cells in cylindrical agglomerate, *dimensionless*

n_{hclc} – contact yield in the designed surface culture of two different strains, *dimensionless*

PA, PB, PC,... – denotations of products (metabolites)

P_f – probability coefficient for bioparticle contacts, *dimensionless*

$P_{f(A\text{-}B)}$ – probability coefficient for heterologous contacts, *dimensionless*

$P_{f(A\text{-}A)}$, $P_{f(B\text{-}B)}$ – probability coefficient for homologous contacts, *dimensionless*

$P_{f(A\text{-}B)nf}$ – probability coefficient for heterologous cell contacts in cell agglomerates, *dimensionless*

$P_{f(A\text{-}B)clag}$ – probability coefficient for heterologous cell contacts in cell agglomerates, *dimensionless*

q, q_1, q_2, q_3,... – proportionality factors of cell contact frequency, $L^3\ T^{-1}$

q_{ms} – specific rate of substrate uptake, $M\ T^{-1}$

q_{ox} – specific oxygen uptake rate, $M\ T^{-1}$

q_{pA} – specific rate of product PA formation, $M\ T^{-1}$

q_{pB} – specific rate of product PB formation, $M\ T^{-1}$

q_{pC} – specific rate of product PC formation, $M\ T^{-1}$

q_{sB} – specific rate of product PA uptake by cells of the strain B, $M\ T^{-1}$

q_{sC} – specific rate of product PB uptake by cells of the strain C, $M\ T^{-1}$

S_{base} – surface area of the base of cylindrical agglomerate, L^2

S_{cyl} – outer layer surface area of cylindrical agglomerate, L^2

S_{ola} – agglomerate outer layer surface area, L^2

S_{tube} – cylinder mantle surface area, L^2

s_{cell} – surface area occupied by particular cell in agglomerate outer layer, L^2

T – time

t – cultivation time, T

V_{agl} – agglomerate volume, L^3

v_{cell} – microbial cell volume or the volume occupied by particular cell in the agglomerate, L^3

W_A, W_B, – weighting factors (contact amplification factors), *dimensionless*

Greek letters

γ – designation of mass concentration

γ_{LO2} – dissolved oxygen concentration, $M\ L^{-3}$

γ^*_{LO2} – saturation dissolved oxygen concentration, $M\ L^{-3}$

γ_{LO2s} – dissolved oxygen concentration in nutrient medium, $M\ L^{-3}$

γ_{pA} – product PA concentration, $M\ L^{-3}$

γ_{pB} – product PB concentration, $M\ L^{-3}$

γ_{pC} – product PC concentration, $M\ L^{-3}$

γ_s – substrate concentration, $M\ L^{-3}$

γ_{s0} – substrate concentration in feeding nutrient medium, $M\ L^{-3}$

δ – dilution rate, T^{-1}

μ_A – specific microbial cell reproduction rate (or specific growth rate) referring to the microbial strain A, T^{-1}

μ_B – specific microbial cell reproduction rate (or specific growth rate) referring to the microbial strain B, T^{-1}

μ_C – specific microbial cell reproduction rate (or specific growth rate) referring to the microbial strain C, T^{-1}

μ_D – specific microbial cell reproduction rate (or specific growth rate) referring to the microbial strain D, T^{-1}

μ_{AT} – specific microbial cell reproduction rate (or specific growth rate) referring to the total cell population of the strain A, T^{-1}

π – 3.14159 +; designation of the ratio of the circumference of a circle to its diameter, *dimensionless*

References

1. W. J. Moore: *Basic Physical Chemistry*, Prentice-Hall International, Englewood Cliffs, New Jersey (1983).
2. H. Roepke, J. Riemann: *Analog Computer in Chemie und Biologie,* Springer, Berlin (1969).
3. M. Bošnjak, *Kem. Ind. 31* (1982) 545–559.
4. C. Patten (Ed.): *Systems Analysis and Simulation in Ecology, Vol. 1*, Academic Press, New York (1971).
5. C. Rayssiguier, D. S. Thaler, M. Radman, *Nature, 342* (1989) 396–401.
6. W. C. Fuqua, S. C. Winans, E. P. Greenenberg, *J. Bacteriol. 176* (1994) 269–275.
7. N. T. Keen, *Nature Biotechnol. 17* (1999) 958–959.
8. S. J. Pirt: *Principles of Microbe and Cell Cultivation*, Blackwell Sci. Publ., Oxford (1975).
9. K. Mihaljević, M. Bošnjak, *Food Technol. Biotechnol. 35* (1997) 89–97.
10. M. Bošnjak, K. Mihaljević: Prolonged fed batch cultivation. In: *Bioprocess Engineering Course*, M. Berovič (Ed.), National Institute of Chemistry, Ljubljana (1998) pp. 344–369.
11. M. Bošnjak, A. Bago-Joksović, Z. Toman, D. Hranueli, K. Mihaljević: Applicability of fortified agarised media in

comparing antibiotic productivities of streptomycetes (ext. abst.). In: *Biotechnology 2000*, Book of Abstracts, Vol. 2, Publ. DECHEMA e.V., Frankfurt/M (2000) pp. 322–324.

12. M. Bošnjak, Š. Šumiga-Kobrehel, E. Gaal, D. Sladić: Fortified agarised media as an efficient tool for improved screening of microorganisms (ext. abst.). In: *ibid.* pp. 325–327.

13. D. Hranueli, J. Pigac, T. Smokvina, M. Alačević, *J. Gen. Microbiol. 129* (1983) 1415–1422.

14. G. Gregorović, D. Vujaklija, *Food Technol. Biotechnol. 39 (2001)* 49–53.

15. E. Bailey, S. Birnbaum, J. L. Galazzo, C. Khosla, J. V. Shanks: Strategies and challenges in metabolic engineering. In: *Biochemical Engineering VI*, W. E. Goldstein, D. Dibiasio, H. Pedersen (Eds.), *Annals of the New York Academy of Sciences, 589* (1990) 1–15.

16. S. Novak, V. Johanides: Impact of biochemical engineering on biomedicine, In: *Current Studies of Biotechnology, Vol.1, Biomedicine*, Z. Kniewald *et al*. (Eds.), Croatian Society of Biotechnology, Zagreb (2000) pp. 45–50.

17. V. Lengeler: Basic concepts of microbial physiology and metabolic control. In: *Bioprocess Engineering Course*, M. Berovič (Ed.), National Institute of Chemistry, Ljubljana (1998) pp. 45–89.

18. M. Bošnjak, A. Stroj, M. Vampola, S. Bošnjak, D. Vlašić, *Kem. Ind. 34* (1985) 39–43.

Uvod u teorijske osnove povećanja vjerojatnosti heterolognih dodira između bioloških čestica

Sažetak

U radu se obrađuju teorijske osnove o pojavi dodira između bioloških čestica i vjerojatnoj učestalosti tih dodira. Analiziraju se činitelji vjerojatne učestalosti različitih dodira glede njihova učinka na moguće heterologne rekombinacije, koje bi mogle dovesti do pojave i rasta mikrobnih stanica s novim svojstvima te mogućom sposobnošću proizvodnje novih biološki aktivnih tvari. Raspravlja se o značenju i učinku morfoloških razlika bioloških čestica te utjecaju metoda uzgoja. Primjenjujući kompjutorsku simulaciju, proučavana je kinetika procesnih zbivanja u hipotetičnoj mješovitoj kulturi dvaju roditeljskih mikrobnih sojeva. Radi provedbe kompjutorske simulacije, razvijen je odgovarajući matematički model. Rezultati dobiveni kompjutorskom simulacijom potvrdili su prikladnost primijenjenog matematičkog modela za opis kinetike rasta roditeljskih i proisteklog mikrobnog soja. Primijenjenim matematičkim modelom mogu se također objasniti kinetike drugih zbivanja u mješovitoj mikrobnoj kulturi. Raspravljajući o značenju pojedinih parametara, posebna je važnost dana biološkom značenju mikrobnih stanica. Sposobnost mikrobnih stanica da svoja svojstva i ponašanje prilagođuju svojem neposrednom okolišu čimbenik je od životne važnosti. Modelom se na prihvatljiv i prikladan način objašnjavaju procesna zbivanja tijekom punidbenog (šaržnog) i kontinuiranog procesa mikrobnog uzgoja.