FOOD PRODUCTION TECHNOLOGY

ANALYSIS OF LIVING AND REPRODUCTIVE PARAMETERS OF MICROORGANISMS

A. A. Maiorov

Siberian Research Institute for Cheese-Making,
Russian Academy of Agricultural Sciences,
ul. Sovetskoi Armii 66, Barnaul, 656016 Russia,
phone: (3852) 56-46-12, e-mail: sibniis.altai@mail.ru

Received August 18, 2013; accepted in revised form September 3, 2013

Abstract: A probability correlation between various transitions and the number of microorganisms at different stages of growth has been analyzed. Comparison of the given parameters with those of the environment (temperature, active acidity, oxidation-reduction potential, etc.) allows defining the influence of each parameter. The obtained results and correlations can be recommended for modeling the growth of microorganisms in different environments, cheese mass being one of them.

Key words: microorganism growth, environment, cheese, cultivation process, optimization algorithm

UDC 637.1
DOI 10.12737/2045

The ability of microorganisms to grow plays an important role in dairy production [1, 2]. The microorganisms, owing to enzymes they produce, impact the texture, smell, and flavor of a dairy product. The probiotic characteristics of such a product play an important role, too. To ensure successful reproduction of microorganisms, appropriate growth conditions must be provided.

Reproductive capacity is best assessed by using the probability theory. In this case, the probability of division of one cell living in specific conditions, characterized by the presence and concentration of a substrate, water activity, active acidity, the salt weight fraction, and a number of other parameters that influence the cell's life, is calculated [3, 5, 7].

This can be done on the basis of either special or previously conducted experiments provided that the conditions of such experiments were recorded. Both methods require compiling a rather large database that helps predict the behavior of bacteria in any given conditions. As complicated as it may seem at first glance, this task requires a strictly formalized approach to the description of the properties of both microorganisms and their environment. The present-day methods of mathematical modeling make it possible to predict the behavior of objects and their interaction with the environment [6, 10].

As regards the growth of microorganisms, a distinction should be made between a closed (uncontrolled) and controlled environment. Partially controlled systems can also exist. An uncontrolled system is such that is not exposed to external influences or when such exposure is negligible. The ideal uncontrolled system is a thermally insulated and hermetically sealed tub containing a substrate with the original number of microorganisms. Nominally, cheese mass at the ripening stage can be considered such a system [11, 13]. The main physical and chemical processes in cheese are influenced by ferments, i.e., chemical components that make up the cheese mass. Microorganisms are actively involved in this process as they take up nutrients, release metabolic products, and change the environment. Their activity during cheese ripening can only be affected by changing the temperature. A decrease in the temperature results in the reduced reproductive rate; an increase in the temperature accelerates the rate of cell division.

The majority of cheeses ripen within a temperature range of 8–20^{o}C. During cheese ripening, its moisture content changes owing to water evaporation off the surface. This content is not large as opposed to the total cheese mass, but it can be of paramount importance as it influences the life of microorganisms.

Therefore, cheese can be referred to a group of systems with partially controlled parameters. In practical terms, it means that the living conditions of microorganisms inside cheese mass can only be controlled by changing its ripening and storage temperature.

The manufacture of fermented milk products is controlled more easily. Fermented milk products are normally manufactured in tanks equipped with a temperature control system (cooling and heating) and agitators. This setup makes it possible to stir the mass during production and influence the temperature. Moreover, various ingredients that influence the living conditions of microorganisms can be added to the mixture. Such ingredients may be salt, sugar, flavoring agents, preservatives, emulsifiers, stabilizers, etc. This system, although isolated from external influences, can be controlled in a wider context. However, the volume of this system and, consequently, its resources are limited, which means that only a certain number of microorganisms can be

grown in this volume. Their maximum concentration is limited not only by the nutrients in the substrate but also by a variety of other factors.

A so-called flow-through fermenter that ensures control over the living conditions of microorganisms (bacteria) is used to produce various biopreparations. In addition to agitators and temperature control tools, such machines are also fitted with a waste products discharge system, a nutrient supply system, and a system that regulates the gas-phase composition supplied to the substrate. This fermenter must be equipped with special tools to control the parameters of cultivation of microorganisms. The main output controlled parameter can be either the volumetric number of microorganisms (biomass volume) or the concentration of waste products produced by microorganisms (ferment). These two indicators do not always correlate with each other. In this event, it is important to have information on how the qualitative and quantitative parameters of the substrate (environment) affect the output parameters (the number of microorganisms and the concentration of the ferment of interest). This information is obtained through special tests by varying environmental parameters and measuring the efficiency of separate and cumulative influence of the environmental parameters. On the basis of the obtained regularities, a cultivation control program is formed to determine the main and supplementary algorithms of cultivation optimization [14, 15].

By analyzing the capabilities of various systems, it is possible to determine ranges of their controllability and build a control algorithm focused on the optimization of the output parameter.

In practical terms, there is a necessity to analyze the dynamics of bacterial flora growth in a given environment. With a high microorganism concentration in a volume unit, the population influence on the chemical composition of the environment is very significant and often plays a decisive role. Special chemostats that ensure steady cultivation conditions can be used for quite an accurate study of the influence of environmental parameters on the growth of microorganisms [12, 13].

When cultivating in a changing environment, it is more difficult to analyze the effect of individual factors, which leads to the ambiguous interpretation of the obtained results. A more detailed picture of the growth of microorganisms in an environment can be obtained using the living environment reconstruction method (LER). Analysis of the dynamics of microflora growth in cheeses is an example of the application of this method.

The growth of microflora in cheese is assessed by the results analyzed at different production stages. As cheese transitions from one stage to the next, it is very difficult to take into account the influence of various factors on both the cheese and its microflora. In reality, as each factor is a time-dependent variable, it is a challenging task to measure a share of influence that each of them exerts on the microflora growth.

Additional information relating to the influence of such factors can be obtained on the basis of the dynamics of changes in the microflora population. For this purpose, time sampling of the microflora growth at given intervals must be conducted. The sampling interval must be proportionate to the period of microorganism generation, for instance, 0.5 h. A differential curve can then be built, which, in its simplest form, is a difference in the microflora population at the previous and the next sampling interval:

$$D = Qi+1 – Qi.$$

Ideally, each cell of microorganisms is divided in two:

$$Qi+1 = 2Qi;$$

i.e., the population of microorganisms doubles at every interval.

In practical terms, not all microorganisms are capable of division.

The division capability is determined by a combination of factors and can be defined as follows:

$$Ki = \frac{Qi+1}{2Qi},$$

where Qi+1 is the number of microorganisms in the next generation; Qi is the number of microorganisms in the previous generation; and Ki is a coefficient that characterizes what portion of microorganisms achieves their capacity to divide.

This coefficient can be interpreted as a cell division probability at interval i. This helps calculate the probability of cell division at every division stage. In this case, it is more accurate to speak not about the cell division probability but about a cell division coefficient at a given stage, which is a cumulative influence coefficient embracing all factors affecting the microorganisms.

When the general influence regularities of each factor on the probability of MO cell division are known, it is possible to determine the share of influence of each factor at various stages.

When analyzing a population change as an elementary process of cell division, the approach based on the assessment of division probability becomes appropriate. As a matter of fact, the reproduction of microorganisms is based on the division of individual microorganisms, and the population growth, on the whole, depends on what portions of the microorganisms will divide. In other words, the division process can be thought of as random or stochastic. A cell transition from being undivided into being divided (two cells) is a discrete process. The probability of division, in this case, is a function of a whole number of factors, a time factor being one of many.

In some cases, this factor can be of paramount importance since normally the microflora growth is described in “number”–“time” coordinates.

The use of random processes to describe microorganism growth allows moving on to criterial assessments, which are very important when studying regularities based on the multistage influence of many factors.

When using deterministic functions, any indicator can be calculated with a 100% certainty by changing its functionally dependent argument; this, however, cannot be applied to cell division. Even when dealing with strictly defined parameters of reproduction environment

and a strictly selected strain of microorganisms, it cannot be stated with assurance that a cell will divide into two cells at a strictly determined interval (for instance, 23.4 minutes). This only means that a cell division process can occur within 22–25 minutes under specific cultivation conditions. In other words, there is a high probability that a cell will divide between the 22nd and 25th minutes of cultivation. In terms of the strict wording, one should speak of the probability of cell division within a given timeframe. A cell division probability curve can be asymmetrical due to the different nature of restrictions which accelerate or decelerate the division process. Variations in the cultivation conditions change both the coordinates of the curve maximum point and the steepness of the ascending and descending slopes. When the cultivation conditions move outside of an optimal zone, the probability decreases; as the distance grows, the probability value becomes more negligible. The envelope of these curves represents a biokinetic zone, i.e., a zone where the existence of microorganisms with a specified probability is possible.

The cell division probability describes an increment or, rather, an increment rate over time. To complete the picture, it is necessary to consider the duration of a cell reproduction age, which can be quite lengthy but not lead to the increase in cell population. Finally, an important element in the overall picture of the growth of microorganisms is the end of their life or the cell death.

Depending on the environmental conditions, microorganisms can stay at each 'stage of life' for a different period. On the whole, life cycle duration for a microorganism can be assessed on the basis of the probabilities for such microorganisms to stay in three main states.

In mathematical terms, it is not quite appropriate to use the system of differential equations to describe the reproduction of microorganisms as it can be used only for continuous functions, whereas the division process in itself, as it has been previously stated, is discrete, i.e. discontinuous.

Speaking about the application of mathematical systems, it is worth noting that the queuing theory along with the Markov chains is an effective method of analyzing the reproduction of microorganisms. [17]

Thus, transition from one state to another can be described by calculating the probability or intensity of transitions. The reproduction process can be represented as a transition from one state to another.

All microorganisms (cells) can be conventionally divided into three groups representing different states. The first group includes microorganisms that are capable of dividing within the timeframe of one generation (the productive category).

The second group includes microorganisms that are presently nonproductive but have the potential to divide at the next stages (the reversible category). This category can be further divided into subcategories depending on their previous history. This category must include cells in an adaptation state after division, cells exposed to mutation or antagonistic pressure from other cells, or cells deprived of sufficient nutrition, etc. These factors can be specified when modeling biochemical and biophysical processes. In any case, it is assumed that cells that belong to this category maintain the potential for future division.

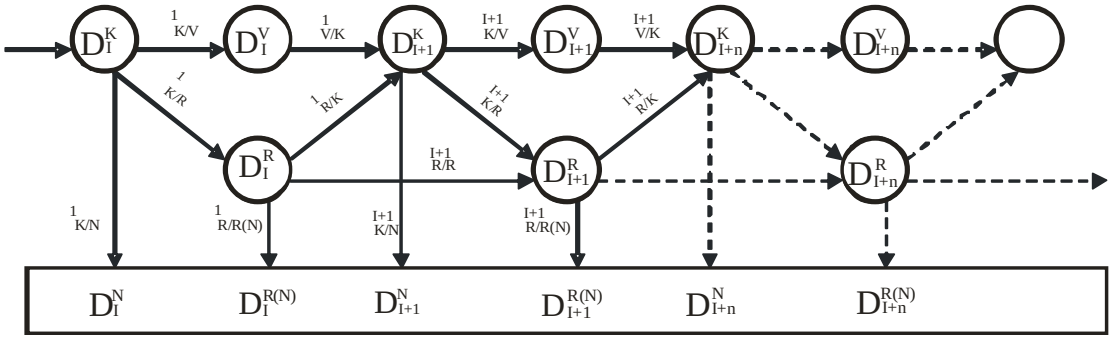

Fig. 1. Pattern of microorganism division.

The third group comprises microorganisms whose reproductive capability is irreversibly lost (the irreversible group). This group cannot be identified by microbiological tests such as inoculation of media but can distort the interpretation of the dynamics of growth of microorganisms when the population is measured by nephelometric or turbidimetric analysis. This subtle detail of using population data should be taken into consideration as it plays an important role in building an accurate model and interpreting test results. Regarding lactate microflora, it is assumed that cells formed as a result of mother-cell division are equal. Accumulation of defects leading to infertility of cells happens with an equal degree of probability for both branches that evolve in the reproduction process. It does not mean a limitless number of cell divisions even when reproduction conditions are favorable. Part of a cell population can be exposed to mutation as a result of errors accumulated during successive generations, and, consequently, take on new properties; the other part loses the capability to reproduce owing to irreversible changes in the genetic apparatus.

When building a mathematical model of population development based on the Markov chains, an overall schematic diagram can be represented by a marked graph that includes all states of the system with the specified transition intensities (Fig. 1). The number of states depends on the complexity of the process model under consideration.

At the initial stage, there is a population containing D_1 of microorganisms. Influenced by a combination of internal and external factors, the population transits into state *V* with a number of productive units equal to D_1^V, thus, distinguishing *N* (irreversible) and *R* (reversible) categories with populations D_1^N and D_1^R, respectively. The transition intensity from state *K* into states *V*, *N*, and *R* is described by coefficients $\lambda^1_{K/V}(\tau)$, $\lambda^1_{K/N}(\tau)$, and $\lambda^1_{K/R}(\tau)$. The value of these coefficients depends on the combination of factors that influence the population. When there exists a probability of reverse transitions, appropriate coefficients $\lambda_{n/i}$ can be used to describe the processes. Each transition may be characterized by certain intensities. In this case, based on the given definitions, part of the reversible category can replenish the productive category in the next reproduction period; the remaining part of the reversible category may transit into the similar category during the future reproduction process.

In terms of the formal approach, the part of the reversible category that transits into the similar category of the next period can be considered as a part of the irreversible category as it plays the same role in population development as the irreversible group under the steady process of changing environmental parameters. However, for building an accurate model and for the correct interpretation of its behavior during research, the transition structure should be kept in the state as it is shown in Fig. 1.

The pattern shown in Fig. 1 can be replaced with a recursive pattern, i.e., repeating itself at every stage of reproduction. Then, as was mentioned before, the reproduction pattern will have five groups of microorganisms (five states). In reality, there are three groups involved in the pattern: productive, reversible nonproductive, and irreversible nonproductive (dying cells). The fourth and fifth groups are made of a hypothetical part of microorganisms consisting of microorganisms in a metastable state and a group that represents a new generation, i.e., a productive group from the previous generation doubled in number.

The state graph for such system is shown in Fig. 2. A group of microorganisms in state S_1 (the metastable state) transits into states S_2 (the reversible group) and S_5 (the irreversible group). Part of microorganisms transits from state S_1 into state S_3 (the productive group). The intensity of transitions from one state to another is characterized by appropriate coefficients λ_i.

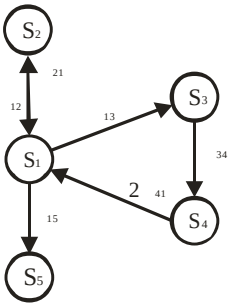

Fig. 2. State graph of the system of microorganisms.

Based on the assumption that the number of microorganisms found in an environment in any state of system S_i is a random value with an exponential distribution function and transition intensity at this stage (λ_i), the reproduction process corresponding to the flow graph can be described by a system of equations:

$$P_1(\tau) = (\lambda_{21} - \lambda_{12} - \lambda_{13} + 2\lambda_{41} - \lambda_{15})P_1(\tau);$$
$$P_2(\tau) = (\lambda_{12} - \lambda_{21})P_1(\tau);$$
$$P_3(\tau) = \lambda_{13}P_1(\tau) - \lambda_{34}P_3(\tau);$$
$$P_4(\tau) = \lambda_{34}P_3(\tau) - 2\lambda_{41}P_4(\tau);$$
$$P_5(\tau) = \lambda_{15}P_5(\tau),$$

where $P_i(\tau)$ is probability that a microorganism at time τ is in state S_i.

By analyzing the correlation between various transition probabilities and the number of microorganisms at different stages of growth and by measuring these parameters against those of the environment (temperature, active acidity, oxidation-reduction potential, etc.), it is possible to determine the degree of influence that each of them exerts. The obtained results and correlations can be used in further modeling the growth of microorganisms in different environments, cheese mass being one of them.

All probabilities can become permanent provided the cultivation conditions remain invariable.

Trial experiments in modeling the growth of microorganisms in a closed uncontrolled environment with a limited supply of nutrients, have proved that a suitable model created on the basis of the approach suggested in this article, is quite possible.

REFERENCES

1. Tutel'yan, V.A., *Novye tekhnologii v nauke o pitanii* (New Technologies in Food Science), Moscow, 2001.
2. Lipatov, N.N., Aspects of modeling amino-acid balance in food products, *Pishchevaya i pererabatyvayushchaya promyshlennost' (Food and Food Processing Industry),* 1986, no. 4, pp. 49–52.
3. Maiorov, A.A., Razrabotka metodov upravleniya biosistemoi syra s tsel'yu sovershenstvovaniya traditsionnykh i sozdaniya novykh tekhnologii (Development of methods of cheese biosystem regulation to improve conventional and create new technologies), *Extended Abstract of Doctoral Dissertation in Engineering*, (Kemerovo, 1999).
4. Osintsev, A.M., Braginskii, V.I., Ostroumov, L.A., and Gromov, E.S., Ispol'zovanie metodov dinamicheskoi reologii dlya issledovaniya protsessa koagulyatsii moloka (Application of dynamic rheology in studying milk coagulation process), *Khranenie i pererabotka sel'khozsyr'ya (Agricultural Commodities Storage and Processing),* 2002, no. 9, pp. 46–49.

5. Shamanova, T.P., Mikrobiologicheskie i tekhnologicheskie podkhody k proizvodstvu fermentirovannykh produktov (Microbiological and technological approaches to fermented milk production), *Molochnaya promyshlennost' (Dairy Industry)*, 1998, no. 3, pp. 18–20.
6. Adler, Yu.P., Markova, E.V., and Grankovskii Yu.V. *Planirovanie eksperimenta pri poiske optimal'nykh uslovii (Planning an Experiment in Search of Optimal Conditions),* Moscow, Mir, 1976.
7. Polishchuk, P.K., Derbinova, E.S., and Kazantseva, N.N., *Mikrobiologiya moloka I molochnykh produktov (Microbiology of Milk and Dairy Products),* Moscow, Pishchevaya promyshlennost', 1978.
8. Babak, O., Ispol'zovanie mikroorganizmov v pishchevoi promyshlennosti (Usage of microorganisms in the food industry, *Produkt BY (Product BY)*, 2008, no. 11. http://www.produkt.by
9. Borunova, S.B., and Furik, N.N., Analiz vyzhivaemosti termofil'nykh molochnokislykh mikroorganizmov pri zamorazhivanii (Analysis of survival capabilities of thermophilic lactate microorganisms during freezing) in *Sovremennye tekhnologii sel'skokhozyaistvennogo proizvodstva: materially XIV Mezhdunar. nauch.-prakt. konf. (Present-day Technologies in Agricultural Production: Proceedings of the XIV International Research and Practice Conference)*, Grodno, 2011, part 2, pp. 273–274.
10. Glazova, A.A., Botina, S.G., and Tipiseva, I.A., Ustoichivost' otechestvennykh proizvodstvennykh kul'tur bakterii roda *Lactobacillus* k antibakterial'nym preparatam (Resistance of nationally manufactured *Lactobacillus* bacteria cultures to antimicrobials), in *Sbornik trudov Moskovskogo gosudarstvennogo universiteta pishchevykh proizvodstv (Collected Works of Moscow State University of Food Industries),* Moscow, 2010, pp. 67–77.
11. Gudkov, A.V., *Syrodelie: tekhnologicheskie, biologicheskie i fiziko-khimicheskie aspekty: monografiya (Cheese-Making: Technological, Biological, Physical, and Chemical Aspects: A Monograph),* Moscow, DeLi Print, 2003.
12. Emel'yanov, S.A., Khramtsov, A.G., Suyunchev, O.A., et. al., Vliyanie temperatury na razvitie mikroorganizmov v moloke i molochnykh produktakh (Temperature impact on microorganism growth in milk and dairy products), *Vestnik SevKavGTU (North Caucasus STU Bulletin)*, 2006, no. 2, pp. 54–57.
13. Knyazhev, V.A. and Tutel'yan, V.A., Mikroorganizmy i pishcha. Risk i pol'za (Microorganisms and food: Risks and benefits), *Vestnik Rossiiskoi akademii meditsinskikh nauk (Herald of the Russian Academy of Medical Sciences)*, 2000, no. 12, pp. 3–6.
14. Holt, G., Krieg, N., Sneath, P., Staley, J., and Williams, S., *Bergey's Manual of Determinative Bacteriology*, in 2 vols., 9th ed., Baltimore, Williams & Wilkins, 1994; Moscow, Mir, 1997.
15. Ostroumov, L.A., Prosekov, A.Y., Kurbanova, M.G., and Kozlova, O.V., Pitatel'nye sredy dlya bifidobakterii (Nutritional environments for bifidus bacteria), *Molochnaya promyshlennost' (Diary Industry)*, 2010, no. 1, pp. 20–21.
16. Prosekov, A.Yu., Kozlov, S.G., and Kurbanova, M.G., Vliyanie fermentatsii zakvasochnoi floroi na nekotorye svoistva molochnogo belkovo-uglevodnogo syr'ya (Influence of starter culture fermentation on certain properties of dairy protein and carbohydrate raw materials), *Khranenie i pererabotka sel'khozsyr'ya (Agricultural Commodities Storage and Processing)*, 2004, no. 9, pp. 31–33.
17. Gorban, A.N., Gorban, P.A., and Judge, G., Entropy: The Markov Ordering Approach, *Entropy,* 2010, vol. 12, no. 5, pp. 1145–1193.