CHANGES IN CULTURE MEDIUM COMPOSITION DURING THERMAL STERILIZATION AND OPPORTUNITIES FOR ITS MODELING

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Abstract*. the modern biotechnology rapidly develops and has many directions.*
Microbial biotechnology production is characterized by common sequentially executed stages and distinctive features.
Preparation stages, receipt of seed materials, production culture growth under surface and depth conditions and final product extraction are common for all productions. However, each specific case has its differential peculiarities.
Differences are manifested in the culture medium composition, preparation methods, production culture cultivation and final product acquisition technique.
Computer technology development has facilitated calculation of biotechnology production equipment by means of computer programs and biotechnological process modeling.
The work deals with the main components of culture medium, and analyses the features of culture medium preparation and sterilization, as well as effective sterilization criteria.
There are considered the prospects of culture medium modeling using the methods of graph theory, as well, that will enable us to determine the changes in external factors action at different variables, to take into account the effect of many factors on microorganisms' growth and on the synthesis of biologically active substances.
Key words*: culture medium, biotechnology, modeling, microorganisms.*

ИЗМЕНЕНИЯ В СОСТАВЕ ПИТАТЕЛЬНОЙ СРЕДЫ ПРИ ТЕПЛОВОЙ СТЕРИЛИЗАЦИИ И ВОЗМОЖНОСТИ ЕЕ МОДЕЛИРОВАНИЯ

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Аннотация: *современная биотехнология быстро развивается и имеет множество направлений.*
Производство в микробной биотехнологии характеризуется общими последовательно выполняемыми стадиями и отличительными особенностями.
Общими для всех производств являются этапы подготовки, получение посевного материала, выращивание производственной культуры в поверхностных и глубинных условиях и получение конечного продукта. Каждый конкретный случай имеет свои отличительные особенности.
Различия выражаются в составе питательной среды, в способах подготовки, в культивировании производственной культуры и в методах извлечения конечного продукта.
Развитие компьютерных технологий поспособствовало расчету установок для биотехнологического производства посредством компьютерных программ и моделированию биотехнологических процессов.
В работе рассмотрены основные компоненты питательной среды, проанализированы особенности приготовления и стерилизации питательной среды, критерии эффективной стерилизации.
Рассмотрены также перспективы моделирования питательной среды с использованием методов теории графов, что позволит нам определить изменения в действии внешних факторов при различных переменных, учесть воздействие многих факторов на рост микроорганизмов и на синтез ими биологически активных веществ.
Ключевые слова*: питательная среда, биотехнология, моделирование, микроорганизмы.*

The modern world focuses more and more attention to the biological processes, which use the vital activity of organisms [1,17].

Intensive development of biotechnology is predetermined by success of biological sciences, especially gene and cell engineering.

Receipt of biologically active substances through microbial synthesis is currently switched to the large-scale production.

Rapidly increased application of biologically active substances of microbial origin has become one of the main preconditions of large-scale production establishment. These substances force out the substances of animal and vegetable origin, as well as products of chemical synthesis.

Receipt of large quantities of microorganisms, which are the source of biologically active substances, is a great deal simpler and economically reasonable, than the preparation of biologically active substances based on the raw animal or vegetable materials.

Many scientific disciplines contributed to the development of biotechnology [1, 11, 12, 17-19].

It is important to provide the hardware support of biotechnological studies that promotes solution of modern-day problems in different direction of biotechnology, including microbial biotechnology, aqua-biotechnology, health-related and industrial biotechnology [1-11].

It should be noted that computer technology development was promoted by the advancement of such prospective field, as bioinformatics, which attaches great importance to the application of computer sciences for data processing on DNA and protein, as well for calculation of biotechnological production equipment and biotechnological process modeling [10, 12].

Any microbiological production can be represented in the form of sequentially executed stages or steps.

Culture medium preparation stages, receipt of seed materials, cultivation of production culture and final product extraction are common for all productions. However, each specific microbiological production has its own distinctive features expressed in culture medium composition and their preparation methods, in cultivation conditions of production culture and in final product extraction technique. But these peculiarities don't change the general pattern of microbiological production [11].

Culture mediums are one of the main factors acting on the rapid growth of microorganisms and their maximum synthesis of various biologically active substances.

When selecting culture mediums, we have to take into account their full value, i.e. substantiated and balanced selection of different nutritive substances, which are necessary for building of growing microorganism cells and for synthesis of final targeted product.

All elements, from which the cell is formed have to be presented in the culture medium for normal growth and development of microorganisms.

Growth of many microorganisms in one or another culture medium requires a whole range of additional conditions: definite concentration of hydrogen ions, reductive-oxidative potential, necessary ratio of different ions.

Some microorganisms with impaired inherited traits (auxotrophic mutants) need special growth factors, including aminoacids, vitamins etc., which can't be synthesized by them.

Culture mediums of different composition are used for receipt of microbiological synthesis products depending on producer-microorganism and production technology. Differently composed culture mediums can be used in a single engineering process for receipt and multiplication of seed material and for industrial cultivation stages.

Sources containing carbon and nitrogen are the main components of culture medium.

Hydrocarbons are the raw materials containing carbon. Hydrocarbons are one of the most important component parts of culture medium necessary for microorganisms' growth. They are used for cellular structure synthesis and at the same time are the energy sources.

Glucose or starch are most frequently used for industrial biosynthesis.

Starch consists of 96-97,5% of polysaccharides. They are originated during acidic hydrolysis of glucose and may be used by those microorganisms, which have amylolytic enzymes synthesis ability.

Biosynthesis of many biologically active substances runs on the culture medium of complex, frequently changeable chemical composition. Nitrogen sources may be represented in them by proteins, peptides or by free aminoacids. During industrial fermentation corn extract, soya flour or yeast hydrolysate are basically used.

Under usual conditions, culture mediums are exposed at the sterilization temperature from 30 to 40 minutes.

Total cycle of heating, holding and cooling is equal to several hours for large fermenters (63 cub.m.) [6, 11].

Method for culture medium cyclic sterilization has some shortage compared to continuous method. First, in comparison to continuous sterilization, during cyclic sterilization the culture mediums are exposed to longer temperature action that deteriorates culture medium quality. In addition, a growing consumption of steam is necessary during culture medium heating period and finally, a cyclic process is more complicatedly subjected to automation.

Based on the above mentioned, the cyclic sterilization methods is currently used for sterilization in the low-volume devices only.

Thermal stabilization causes some chemical shifts in the culture medium composition. Sometimes, the decay of compounds unstable to heating takes place that leads to the loss of substances necessary for microorganisms' nutrition. Among different kinds of changes one may mention interaction of different components of the culture medium and, in particular, interaction between amino compounds and hydrocarbons that causes formation of products inhibiting microorganisms growth. The major part of changes in culture medium chemical ingredients occurs at temperature higher than sterilization temperature.

Thus, an effective sterilization with minimum environmental changes may be obtained with the use of higher temperature and via rapid heating and cooling.

In the cyclic systems this result is provided by means of indirect steam, which crosses coil pipe or heating jacket, as well as by live steam, which is injected through the nozzle for seeding, air delivery and sampling.

Treatment with a live steam causes formation of condensate. In this regard, one has to take into account medium dilution by condensate and make proper adjustments in culture medium composition. In this case, in the end of sterilization we will have necessary concentration of nutritional ingredients.

If hydrocarbons sterilization will be conducted separately, and then we will add aseptically sterilized medium preliminary prepared on a separate basis then we can avoid the reaction between hydrocarbons and other composite components of culture medium. The components, which are very sensitive to heat action, can be separately sterilized, as well. At the same time, irradiation also may be used for sterilization.

So, as we mentioned above, the short-term sterilization has its own advantages, since at that the culture medium properties are deteriorated to the minimum extent without any degradation of sterilization efficiency. This method can be implemented in the flow, when using continuous sterilization system [6,10,11].

High-temperature short-term sterilization in the flow is used in the systems of continuous sterilization, as a method, which enables us to reduce to the minimum the deterioration of medium nutritive properties without any degradation of sterilization efficiency. In this case the medium holding time rapidly increases at maximum temperatures, while heating and cooling time doesn't exceed several seconds.

For such medium, which is free from suspended solid particles, temperature maintaining at 150-160℃ secures maximum sterilization. Chemical changes taking place in the culture medium at that time are so insignificant that one can neglect them. If there are solid suspended particles in the culture medium, the optimum sterilization temperature has to have lower value, since some additional time is necessary for heat entry inside such particles. This value is determined by the nature of suspended particles, as well as by degree of initial changes in culture medium composition in each isolated case. Sterilization temperature equal to 135°C is considered as the most widespread one, while holding time varies from 5 to 15 min.

In all cases of continuous sterilization, the culture medium cooling is practically implemented in the double pipe (pipe-in-pipe) heat exchanger or in counter-flow plate heat exchanger, while heating is conducted via live steam injection.

In order to fully study the changes in culture medium state due to action of different factors one can create the mathematical model of culture medium. In this case it would be fruitful to use the knowledge accumulation method in the form of structural sequences and to represent it like elementary relation enabling us to construct complex systems [12,14].

Design of the structural study algorithm of modelled system makes it possible to determine the culture medium system structure as "Input-State-Output" model and to study properties change and management, to explore at which variable the external factors change will be revealed first (e.g. high and low temperature action), to determine different hierarchical levels of the system, and to single out sublevels connected at each level and their interrelation [13, 15].

In addition, the analysis of obtained results enables correction of initial hypotheses and assumptions.

The above mentioned circumstance can be successfully used regarding certain chemical changes in the culture medium composition during thermal sterilization. So, we deem that the use of the graph theory is very prospective.

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