

## Economic fermentative unit for production of microbiological means of protection of plants

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**The purpose.** To develop fermentative unit on the basis of the proved technical and technological ways of reduction of expenses in production of microbiological products on thin-walled fermenters. **Methods.** The comparative analysis, assessment of technical and economic parameters, prototyping and experimental researches of the equipment. **Results.** New unit is developed which consists of the modernized industrial autoclave for preparation and sterilization of concentrates of nutrient medium, and two thin-walled fermenters, equipped with air bubblers, and circulating pump. Sterilization of equipment is replaced with disinfectant washing; system of pipelines is made of releasable hoses which are switched by the operator depending on technological operation. Sterilization of air and water is carried out by mechanical filters and ultra-violet irradiation. **Conclusions.** The unit is developed of industrial fermentation for biolaboratories of protection of plants of Ukraine which provides essential reduction capital and working costs.

**Key words:** *thin-walled fermenters, split design, disinfectant washing.*

Biologization of agriculture as a strategic direction of sustainable development of Ukraine provides for widespread use of microbiological means of plant protection against pests and diseases. In the post-Soviet countries, it has historically been found that the production of these means, despite the presence of the microbiological industry, was carried out mainly in biolaboratories that were part of the agricultural system. In other developed countries, such production is concentrated in large corporations with a global structure.

Currently in Ukraine there are dozens of small private enterprises operating in this field, where 10 - 20 employees. They are located in rural areas in adapted buildings, have in fact, they are handicraft production, but they provide biological products with crop production and have all the grounds for further development, requiring appropriate scientific and technical support. The problem is greatly complicated by the unfavorable economic situation in the country, when there are practically no conditions for long-term investments. Therefore, the innovative development of the production of bio-defense products now requires a dramatic increase in the cost-effectiveness of used equipment and technologies.

**Analysis of recent research and publications.** Microbiological production as an industry industry, has a significant and sufficient scientific provision [1]. Its main processes - sterilization and fermentation have many hardware and technology implementations [2]. However, they all, as practice shows, is nothave found application in biolaboratories because of high cost of classical high pressure fermenters, considerable expenses for thermal sterilization and, most importantly, the inexpediency of creating in rural areas mini-factories with complete infrastructure. Therefore, production began on the laboratory equipment, the most effective element of which was KPM-36 rocker, created in the Engineering and technological Institute "Biotechnics". Then the Institute developed so-called thin-walled fermenters without excess pressure, basic ideas that were used during creation most of the productions in Ukraine. These devices are constantly being improved [3, 4]. At their base a modern automated fermentation complex has been created, which has undergone state acceptance tests [5].

Disadvantages of thin-walled devices - difficulties during sterilization of equipment and environments. Against the background of existing approaches of classical microbiology, as strictly as necessary, strictly aseptic production [1], several methods of sterilization have been developed. But the analysis of the experience of operating industries shows that in most cases arbitrary aseptic production [6]. This is due to first of all, the commercial form of microbial preparations, when the sold product is a culture fluid from fermenters, which is packed in plastic containers. Shelf life of drugs - 1 - 2 months. Production is made to order and preparations should be used immediately after purchase. Therefore, appearance and development the extraneous microflora can not worsen normalized quality of the goods.

This practical approach non-discussed in scientific publications. Found only materials that are listed for such features small-tonnage production in thin-walled reactors, "as the development of microorganisms in insufficiently sterile conditions" [7].

In the world, the innovative development of small-tonnage production over the last decade has been through the introduction of single-use fermenters (Single-Use Bioreactors) [8]. They represent a plastic bag in which fermentation is carried out. The bag is supplied sterile, used once, it is equipped with the necessary devices to provide fermentation processes. This eliminates on-site washing and sterilization processes (CIR / SIR), which is a major problem in traditional equipment.

Capital expenditures in one-time systems are reduced, and the cost of a large number of bags significantly increases operating costs. In [9], a useful analysis of the cost of transition to one-time systems was conducted and the numerical data we used to evaluate the effectiveness of these systems in the biolaboratories of Ukraine was provided.

The minimum capacity of the biolaborator is from 36,000 liters of the drug, which at its average price of 40 UAH / liter gives a gross annual income of 1.44 million UAH or 55.4 thousand USD. This power is provided by a 500 liters fermenter for 90 cycles per year.

Capital costs of a variant with a traditional stainless fermentor will be 360,000 cu, which are obtained from the price of a fermenter of 90,000 USD and a factor of Longe [9]  $K_v = 4$ , which reflects an increase in capital costs of commissioning,

Capital expenditures for a disposable fermentor (for the purchase of its building) will amount to 4200 thousand USD, the cost of a one-time sack 500 liters - 543 USD, which, at 90 cycles, will require an extra year - 48 870 USD, which gives 53 thousand USD to the capital. Comparison of annual expenditures of 53 thousand USD at an income of 55.4 thousand USD indicates a complete ineffectiveness of the option of a one-time system, not to mention the traditional fermentor at 360 thousand USD.

The conducted analysis shows the economic problems of using modern foreign technology for the production of microbial preparations at this stage and makes the relevance of further enhancement of the efficiency of fermentation complexes based on thin-walled stainless steel devices.

The purpose of the research is to develop a complete fermentation plant based on sound technical and technological methods to reduce the cost of producing microbial preparations on thin-walled fermenters.

**The purpose.** To develop fermentative unit on the basis of the proved technical and technological ways of reduction of expenses in production of microbiological products on thin-walled fermenters.

**Research methods** The comparative analysis, assessment of technical and economic parameters, prototyping and experimental researches of the equipment.

**Research results.** On the basis of previous research and development work at the Engineering Technology Institute "Biotechnics" [10] a new hardware and technological scheme of the plant and its devices was developed, in which the following basic measures to increase the cost-effectiveness of production were implemented:

- refusal of thermal sterilization of equipment and water;
- constructive maintenance of processes of washing and disinfection of equipment;
- reasonable choice of electrical equipment with minimal power.

Installation of production fermentation (IPF) (Fig. 1) consists of a sterilizer universal SU, two identical fermenters FP1 and FP2, blocks of air preparation BA and water BG.

*Universal sterilizer SU* is made on the basis of the widely used steam sterilizer BK-75-01 with preservation of all its characteristics. The refinement of the factory was carried out by means of external technological strapping. The sterilizer is intended for preparation of a concentrate of a nutrient medium and its thermal sterilization, and also allows to carry out such additional operations, as steam sterilization of different devices or carrying out of fermentation of a mother-alone culture in volume of 55 l.

*Fermenter productive FP* (Fig. 2) provides a deep fermentation with a combined method of energy input: bubbling and mixing at the expense of the external circulating circuit. Fermentation takes place through simplified rules for ensuring the sterility of the process, in particular, with the replacement of the process of thermal sterilization of equipment and pipelines by washing disinfectant solutions.

The fermenter consists of a tank 1 with an easily removable one cover 2. The nutrient medium concentrate is fed from the sterilizer through a flexible hose 8 through the valves B6 and B5. Sterile water for dilution of the concentrate of the nutrient medium is fed through the valve B1, its volume is controlled by a PIC water meter in the water treatment unit of the BG. Sterile air for aeration is fed into a bubbler 6, which consists of a removable vertical riser and bubble tubes; air passes through the filter 5. The release of the finished product and the drainage of the washing solutions takes place through the valve B4.

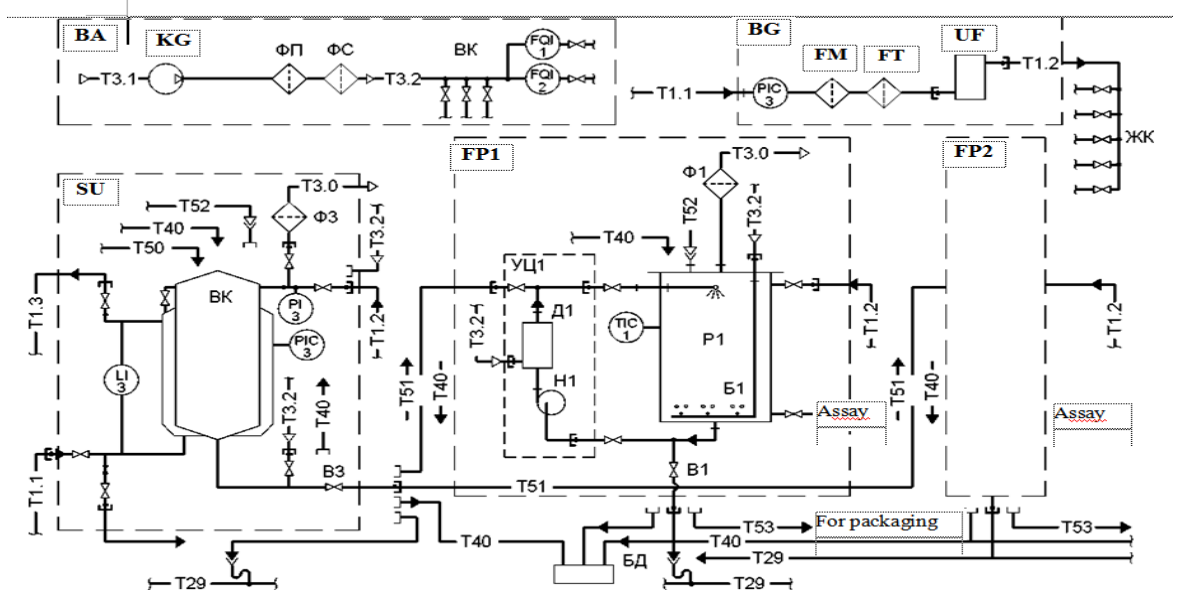


Fig. 1. Hardware and technological scheme of the installation of production fermentation: FP1, FP2 - fermenter; SU - universal sterilizer; BA - air preparation unit; BG is a block water preparation; substance in pipelines: T1. (1, 2, 3) - drinking water, sterile, heated; T3. (0, 1, 2) - the air is worked out, atmospheric, sterile; T29 - sewage; T40 - solution is disinfectant; T50, T51 - nutrient medium - components, concentrate sterile; T52 - sowing material; T53 - culture fluid

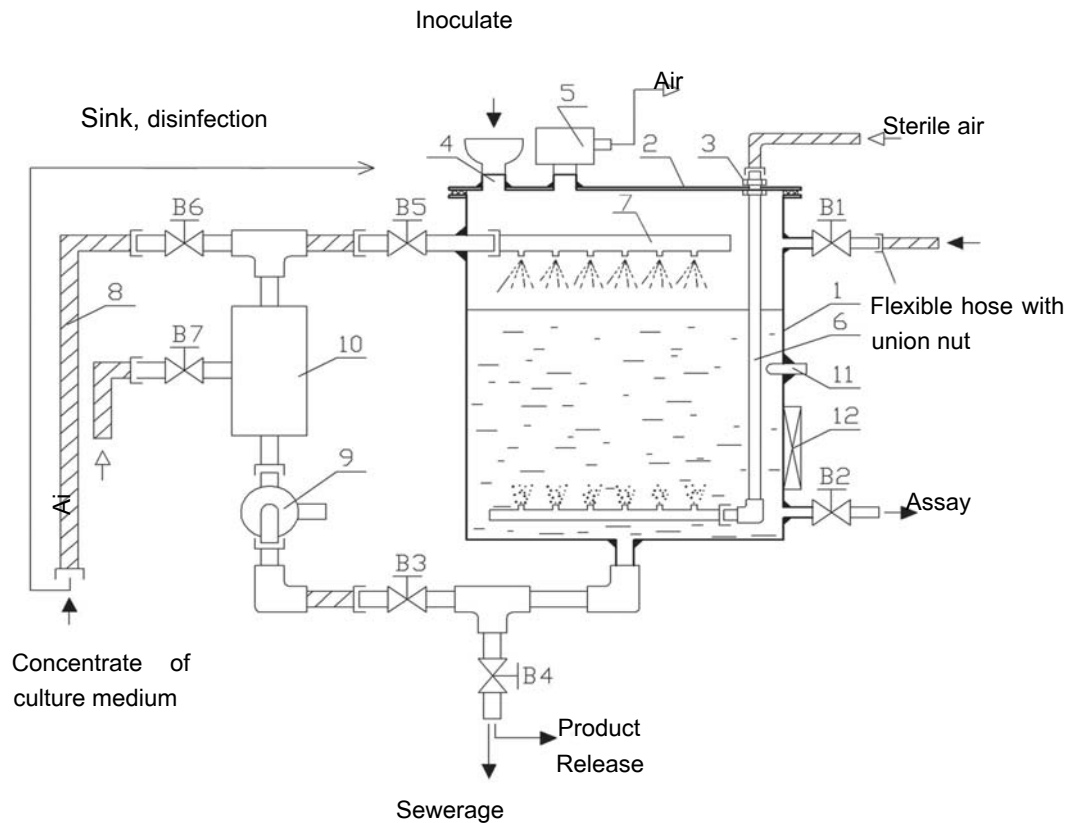


Fig. 2. Technological scheme of the fermenter: 1 - tank; 2 - cover; 3 - detachable retainer; 4 - cap sleeve; 5 - filter graduation; 6 - bubbler; 7 - spray device with nozzles; 8 - flexible hose; 9 - circulation pump; 10 - processing unit; 11 - temperature sensor; 12 - electric heater; B1-B7 - the valve

The fermenter has an external circulation circuit with pump 9 and a spray device 7 with nozzles for aeration and mixing. A modern low-power pump has been selected. The pump is also used to wash the tank after its partial filling with a cleansing solution through circulation or through a hose 8 with a closed valve B5 and an open cover 2. In the processing unit 10 (available for an additional order) emissions of air are carried out through mechanical filter, as well as possible heating (cooling) of the liquid in the flowing exchanger.

Surface electric heater 12 and sensor 11 (Fig. 2) are part of the system automatic temperature control of the liquid in the TIC tank (Fig. 1).

The design of the fermenter provides for its complete parsing, all parts are easy to remove, which simplifies washing and sterilization.

The block of air preparation BA is realized on the basis of standard cartridge filters. In the previous filter, a polypropylene thread is used, in a sterilizing one - a PTFE membrane or its analogues. Modern compressor with a reduced capacity of 3 - 5 times the capacity compared with professional microbiological equipment was used.

The block of water preparation BG is implemented on typical devices. Drinking water under the pressure of the main line undergoes mechanical purification polypropylene FM filter and fine purification in the coal filter FT. Disinfection occurs in ultraviolet irradiation UF.

The main feature of the installation is the lack of a stationary piping system that unites the devices into a single installation. Transportation of all liquids is carried out under the influence of excess pressure of the compressor KG on the removable hoses, which are connected by the operator for each technological operation, for which the valve B3 has 4 variants of connection, valves B1 and B2 - 3 variants. Hoses have a "American" type connector and are included with the package, but they are typical products and can be changed by the consumer.

Fully disassembled piping system, removable covers of fermenters and other parts, as well as their sizes up to one meter, ensure compliance with the requirements of periodicity, disinfection and sterilization.

The installation consists of five constructively independent main apparatuses. SU, FP1, FP2 are installed on supports at the height of 0.4 m above the floor level. Blocks of BA and BG are recommended to be installed on the walls of the room. The weight and dimensions of the knots make them easy to move through standard doors. To accommodate the IPF a production area of 18 m<sup>2</sup> is required.

The productivity of the plant is 440 liters of biological preparation per cycle of cultivation. The advertising cost of the installation IPF - 440 — 19500 USD.

### Conclusions

An analysis of innovative solutions for the creation of equipment for the production of microbial drugs for the protection of plants under the conditions of regional biolaboratory in Ukraine determines the economic benefits of constructing fermentation complexes based on thin-walled reactors with a simplified sterilization of equipment.

A new fermentation plant was developed, in which the sterilization of the equipment was replaced with disinfectant washing, the piping system was made of easily separated hoses, which are commutated by the operator depending on the technological operation.

The installation has compact dimensions for placement in typical laboratory rooms. Its cost is almost twice less than the analogues based on disposable fermenters.

### References

1. *Sydorov, Ju.I.* (2008). *Procesy i aparaty mikrobiologichnoi' ta farmacevtychnoi' promyslovosti* [Processes and apparatuses of microbiological and pharmaceutical industry]. Lviv, 736.
2. *Fedoseev, K.G.* (1969). *Processy i apparaty biotekhnologii v himiko-farmaceuticheskoj promyshlennosti* [Processes and apparatuses of biotechnology in the chemical and pharmaceutical industry]. Moscow: Medicine, 199.
3. *Dobrov, V.I., Kosoy, S.M., Starchevsky, I.P.* (2003). Thin-walled fermentation apparatus. Declaration patent of Ukraine for invention. A61L 2/04, C12M 1/12. № 52896; declared 28.09.2001; published 15.01.2003, № 1.
4. *Kosoy, S.M., Ovcharuk, V.S., Starchevsky, Yu.I.* (2008). Thin-walled fermentation apparatus. Patent of Ukraine for invention. A61L 2/04, C12M 1/12. № 82696; declared 08.12.2005; published 12.05.2008, № 9.
5. *Krutjakova, V.I.* (2015). Kompleks fermentacijnyj modul'nogo typu [Fermentation complex of modular type KFM-420] *Agrarian science - for production*, 4, 26.
6. *Viestur U.Je., Shmite, I.A., Zhilevich, A.V.* (1987). *Biotehnologija: Biologicheskie agenty, tehnologija, apparatura* [Biotechnology: Biological Agents, Technology, Equipment]. Riga: Zinatne, 263.
7. *Kotljarov V.V., Sedinina, N.E.* (2014). Osobennosti malotonnazhnogo proizvodstva mikrobiologicheskikh preparatov dlja zashhity rastenij i ego optimizacija [Features of low tonnage production of microbiological preparations for plant protection and its optimization]. *Scientific journal of the KubSAU*, 100 (06), 9-21.
8. *Sydorov Ju.I.* (2010) Odnorazova fermentacijna aparatura [One-time fermentation equipment] *Biotechnology*, 3, 6, 9-21.
9. *Barak By., Barnoon, I., Bader, Bob.* (2008). Lifecycle Cost Analysis for Single-Use Systems. Less complex single-use systems have more favorable lifecycle economics. *BioPharm International Supplements*, 7, 30.
10. *Hodorchuk V.Ja., Kosoj, S.M.* (2012). Doslidzhennja eksperimental'nogo ustatkuvannja z virobnictva mikrobiologichnih zasobiv biologizacii zemlerobstva [Research of experimental equipment for the production of microbiological means of biologization of agriculture]. *Bulletin of Agrarian Science of the Southern Region*, 12-13, 150-155.