ENGINEERING DESIGN OF DISPOSABLE BIOREACTOR FOR CULTIVATION OF MICROBIOLOGICAL RODENTICIDE ON GRAIN

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Abstract. The equipment and technology were considered for the production of wet grain biopreparation of a bactericidal rodenticide, based on the bacteria *Salmonella enteritidis var*. A means of combating mouse-like rodents in crop production is *Issatchenko.* The object of the research is the technological process and equipment for the stage of cultivation of the preparation on grain, which is usually conducted in glass or metal containers of up to 3 liters. A new solution is proposed of designing a disposable bioreactor with replaceable plastic bags, which corresponds to global trends in the development of small-scale fermentation production. Using the method of an engineering design, fundamental technological and technical solutions have been developed for the process of cultivating bacteria on grain, which ensure an increase in productivity, improved quality of the product, and comfortable working conditions for the personnel. A design was worked out, models of disposable bioreactors for fermentation of bacteria on grain were manufactured and studied in a metal container with a replaceable polypropylene bag for 2 kg of bactericidal rodenticide. By means of the bioreactor all the technological operations are performed from sterilization to packaging of the finished products, the indicators of the technology and bactericidal rodenticide exceeding the standard values. A set of equipment has been identified at the grain stage for the production of 512 kg of bactericidal rodenticide per day.

Keywords: *Salmonella*, grain, designing, fermentation, disposable bioreactor.

Statement of the problem

Biologization of plant growing in modern conditions is a priority area of development of the industry and, to a large extent, is based on the biological method of the plant protection from pests, including mouse-like rodents [1]. An efficient bacterial preparation, based on the strains of microorganisms *Salmonella enteritidis* var. *Issatchenko* (hereinafter *Salmonella*) is used against them. More than 100 years of its use have confirmed its absolute safety for the environment, as well as for non-target warm-blooded animals and humans. The preparation is wet wheat grain, infected with these bacteria, with a concentration of microbial cells in 1 g of bactericidal rodenticide (measured by the number of colony-forming units CFU) of not less than $2 \cdot 10^9$ CFU$\cdot g^{-1}$ according to the existing technical conditions [1]. Application of the preparation up to 3 kg per 1 ha leads to the death of 75% to 95% of rodents.

The preparation is produced in biolaboratories according to the technology and equipment [2]. For culturing *Salmonella* they mainly use 3-liter glass jars, which often burst, or 2-3-liter aluminum cans. Such cultivation containers have significant drawbacks that make it difficult to obtain guaranteed high-quality products. When inoculating, taking samples for analysis, or when packaging bactericidal rodenticide, it is necessary to open an excessively large hole (more than 8 cm in diameter) in each container. This creates conditions for contamination of the bactericidal rodenticide by contaminating microbiota from the air, as well as limits the equipment productivity to 200 kg of the preparation per day. But, despite the above-mentioned shortcomings, such a technology and equipment remain the most widespread at present [3].

The KPM-36 suspended microbiological rocker is widely used for growing *Salmonella* seed cultures of the first and second generations, and the cultivation unit for growing on grain, still used for the cultivation in 2-3 l containers (Fig. 1). A set of equipment for the production of the bactericidal rodenticide BAK-2 [3] (Fig. 2) was also created, in which the cultivation containers were made of 40-litre aluminium cans. Due to the air bubbling the cultivation processes are significantly accelerated. However, the productivity of the set of 100 kg per cycle and the need to move loads over 20 kg make it difficult to use for the organization of production with higher productivity.

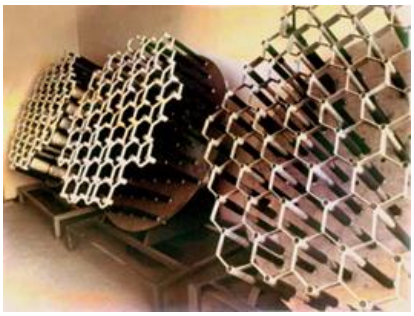

Fig. 1. **Cultivation unit**

An analysis of the problems of growing *Salmonella enteritidis* on grain shows that they are primarily due to the design of the fermentation tank. A possible solution, as evidenced by global practice in the development of biopharmaceuticals, is the use of the so-called disposable plastic bioreactors [5, 6]. The market needs, particularly of the developers and manufacturers of medicines, vaccines and other biological products, determine the trend towards the use of single-use seed bioreactors, as well as production bioreactors. Numerous investigations, confirming their advantages in use, have encouraged further new developments and led to the emergence of many types of disposable bioreactors, differing in the power consumption, design, equipment and scale of the cultivation containers [7]. However, it is noted that the use of disposable bioreactors becomes economically viable only for the production of high-value products due to the high cost of the consumable materials [8]. Bacterodencide does not belong to such products, which is probably why their use for the fermentation of *Salmonella enteritidis* on grain has not been found in publications. Accordingly, to implement this idea, solutions will be required that significantly reduce the cost of the disposable bioreactor. Therefore, the aim of the work is to design a disposable bioreactor for the cultivation of the microbiological rodenticide *Baktorodenkid* on grain in small-scale production, on the basis of which it is possible to create a high-performance set of equipment with the required level of cost-efficiency.

Materials and methods

Technological regulations and guidelines for the production and use of the wet grain bactericidal rodenticide [2]. This technology includes the following stages: reproduction and storage of the *Salmonella* bacteria culture, obtaining the first and second generation seed culture, and growing the bacteria on grain. The final stage involves preparing the grain and growing the bacterial culture in a disposable bioreactor.

As the methodological basis for their rearing were chosen modern approaches to the system design [6; 7]. Our methodology of designing the equipment for the production of biological plant protection means [8] includes the construction of functional diagrams, definition of the design objects, justification of the requirements, synthesis of options, evaluation and selection of the best option.

The object of designing is a disposable bioreactor. The basic requirements: productivity up to 500-1000 kg of bactericidal rodenticide per day, compatibility with the equipment, minimal cost, comfortable working conditions. In Ukraine, manual female labour is used, the maximum load weight is 7-10 kg. Mechanization requires capital expenditures, which is no unacceptable for small-scale production. Analysis of the equipment shows that steam sterilizers are the most expensive. Autoclaves VK-75 and GPD-400 have volumes of 75 and 400 dm^3. The productivity of the bioreactor on VK-75 is 100-200 kg per day, on GPD-400 – 500 kg in two shifts. The main design results were verified. Models were made and tested in real processes. The concentration of microbial cells was determined in dilutions of the bacterial suspension, using a Goryaev chamber or the Koch method [9]. To calculate the concentration of *Salmonella* in grain, a sample of the bactericidal rodenticide was ground and the number of $CFU \cdot g^{-1}$ was determined, using the Koch method [10]. To determine the purity of the culture, the colony characteristics were observed and Gram-stained smears were made [10].

Results and discussion

The design option for the bioreactor, selected at this stage of the work, is shown in Fig. 3. It formed the basis of the created prototypes, which were then used to study the main operations of the sub-stage

of growing the bacterial culture *Salmonella* on grain. As the basis there is chosen a rectangular package 4 (see Fig. 3), measuring 200 × 550 mm, made of biaxially oriented polypropylene with a thickness of 40 µm. The package is placed in container 5. It is a pipe with a diameter of 128 mm with a perforated bottom, made of galvanized steel with a thickness of 0.3-0.5 mm. The bag is filled with moistened grain 6; the lower part of the bag takes the form of a cylinder with a diameter of about 127 mm and a height of about 220-240 mm. After filling, the edge of the bag is pressed around the metal tube-neck 2 using several layers 3 of a heat-resistant nylon tape 10-15 mm wide. The tube, up to 1 mm thick, has an internal diameter of 25 mm, a length of 55 mm, and it is then closed with a cotton-gauze stopper 1.

The manufactured model of the bioreactor is shown in Fig. 4. The neck of the cultivation bag is shown at the moment when assembled manually by a laboratory technician. The neck is then manually pressed down to 400 mm like an accordion to reduce the overall height of the bioreactor. This operation is demonstrated in Fig. 5, where the bags are shown without containers. In this way the **"**dead**"** zone, not occupied by the grain, is minimized to reduce the volumes during their sterilization in the autoclave and in the cultivation unit.

The technological process, using a bioreactor, consists of the following operations: 1 – placing the bag in a container, 2 – packing grain into the bag, 3 – assembling the cultivation bag, 4 – placing the bioreactor in the autoclave, 5 – sterilization, 6 – unloading and cooling, 7 – inoculation of the seed culture through the neck of the bag, 8 – installing the bioreactor on the cultivation unit, 9 – growing *Salmonella* on grain, 10 – removing the bag from the container, sealing and cutting the bag, 11 – packing the bag into a retail package.

Fig. 2. **Steam sterilizer GPD-400**

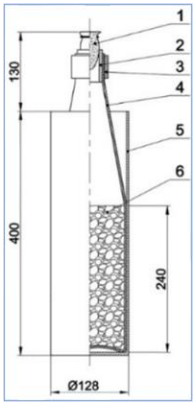

Fig. 3. **General view of a disposable bioreactor:**
1 – cotton-gauze plug; 2 – tube–neck;
3 – heat-resistant tape; 4 – polypropylene bag;
5 – container; 6 – wheat grain

Fig. 4. **Models of a disposable bioreactor and container**

Fig. 5. **Assembled cultivation bags in folded and unfolded states**

The size of the bioreactor and the weight of grain are the determining indicators that directly affect the technical and economic efficiency of production. An obvious requirement is the maximum increase in the grain mass to the values determined by sanitary and hygienic and/or technological restrictions. It is well known that in autoclaves sterilization of the medium in containers with a diameter of 10-13 cm at 121 °C occurs in 1 hour, and with a diameter of 18-20 cm – in 2-3 hours. Therefore, in order to maintain the existing sterilization duration in the common culture vessels, a bioreactor diameter of up to 13 cm with a corresponding grain mass of 2 kg was selected. The working chamber of GPD-400 (Fig. 2) contains 32 horizontally located bioreactors. There is a sufficient gap left between them, which allows hot steam, which is the main factor in sterilization, to fill evenly the entire volume around each container. Therefore, the heat exchange intensity is maintained at the level of the aluminium cans with the grain sterilization duration of 1.5 hours at 127 °C, which ensures 4 autoclave loadings per shift and, accordingly, the output of 128 bioreactors with 256 kg of the bactericidal rodenticide. For two shifts the daily productivity will be 512 kg. After cooling and inoculation the bioreactors are installed in the cultivation unit (Fig. 1), where they are periodically shaken and rotated around an axis. 128 bioreactors require two cultivation units for placement. For further increase of productivity to 1028 kg, this set needs doubling.

During the designing process other variants of containers and cultivation bags were worked out and researched. Initially a multi-seat container seemed promising for increasing productivity. A container for 10 plastic cultivation bags, holding 1 kg of grain, was developed and manufactured, see Fig. 6. The design and dimensions of the container are calculated to accommodate 3 pieces in a VK-75 autoclave with a round vertical chamber. This allows 90 kg of grain to be sterilized in three loads. All microbiological parameters of the process were satisfactory. But, to operate them, two men and a new cultivation unit were required. This confirmed our previous analysis regarding comfortable working conditions and justified the adopted technical solution (Fig. 3).

Fig. 6. **Model of a multi-seat container for 10 bags**

The verification procedure, i.e. the experimental determination of the compliance of the main indicator of the bioreactor with the established standards or technical conditions, was performed by implementing all the operations of the technological process, using manufactured models of disposable bioreactors.

The experimental determination of the bioreactor parameters and the parameters of technological operations with it was accomplished by implementing a complete technological process, using ready-made models. The tests were organized in production conditions. The GPD-400 sterilizer and the cultivation unit were used for their intended purpose. The dosing of grain and seed crops was carried out by installing the bioreactor on electronic scales.

The concentration of the second generation *Salmonella* seed culture was $2 \cdot 10^9$ cells$\cdot$ml^{-1}. The tests were conducted with various amounts of the seed culture, which was introduced into each bioreactor with a grain mass of 2 kg. Each experiment was made on four bioreactors. The results are shown in Table 1.

Table 1 shows the values of the average concentration of bacteria and the confidence intervals. The calculation of the intervals was performed using the Student coefficient t = 3.2 for four replicates with a confidence probability of 0.95.

Table 1

Effect of *Salmonella* quantity of the seed crop upon the bactericidal rodenticide concentration

Amount of seed crop, g	**Concentration of *Salmonella* bacteria in bacteriodencid during cultivation, KUO·g^{-1}**	
	24 hours	48 gours
200	$(7.15 \pm 0.92) \cdot 10^6$	$2.0 \cdot 10^8$
300	$(5.70 \pm 1.51) \cdot 10^9$	$5.5 \cdot 10^9$
400	$(3.65 \pm 0.12) \cdot 10^8$	$2.0 \cdot 10^9$
600	$(2.15 \pm 0.41) \cdot 10^8$	$2.0 \cdot 10^9$

Thus it was established that the optimal amount of the seed crop is 300 g, which is 15% of the grain mass. In addition, the concentration of finished bactericidal rodenticide is $(5.70 \pm 1.51) \cdot 10^9$ CFU·g^{-1}, which exceeds the standard value of $2.0 \cdot 10^9$ according to the technical specifications [1; 3]. This confirms the viability and prospects of the proposed project.

The cultivation time on grain was achieved in 24 hours, which corresponds to the regulatory requirements of 24-48 hours, but is very rarely achieved in the existing technologies. Therefore, after 24 hours the experiment that had been carried out in four replicates was stopped, and one sample with the average indicator was left (Table 1). During the experimental production the presence of contaminating microbiota in the bioreactor grain was repeatedly checked, yet not detected.

Conclusions

There are considered the equipment and technology for the production of wet grain microbiological preparation of a bactericidal rodenticide, based on the bacteria *Salmonella enteritidis* var. *Issatchenko*, which is an effective means of combating mouse-like rodents in the crop production. By the method of engineering design, fundamental technological and technical solutions have been developed for the process of bacteria cultivation on grain, which ensure an increase in its productivity, comfortable working conditions for the personnel, improved product quality, and economic profitability of the innovation.

There has been worked out a design, models of disposable bioreactors manufactured and studied for fermentation of *Salmonella* on a grain medium in the form of a metal container with a replaceable polypropylene bag for 2 kg of bactericidal rodenticide, which eliminates contamination by foreign microflora. Besides, during a 24-hour cultivation period the concentration of bacteria in the finished bactorodencid reaches $5.7 \cdot 10^9$ CFU·g^{-1}, which exceeds the standard values. By means of the bioreactor all the technological operations are carried out from sterilization to packaging of the finished products in one cultivation bag, whereas the indicators of the technology and bactericidal rodenticide exceed the standard values.

The disposable bioreactor is designed for joint use with the common technological equipment, in particular the GPD-400 autoclave, which ensures the modernization of the existing production of bactericidal rodenticides. Its use is also economically justified for the design of a basic set of the equipment with a capacity of 512 kg of the bactericidal rodenticide per day.

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