# TECHNICAL AND TECHNOLOGICAL SOLUTION FOR SMALL-SCALE PRODUCTION OF LIQUID MICROBIOLOGICAL RODENTICIDE IN FERMENTER

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**Abstract.** The most effective and safe method of controlling rodents in agriculture is the use of microbiological preparations based on the bacterium *Salmonella enteritidis* var. Issatchenko. Cultivation is mainly carried out in glass or metal vessels with a capacity of 2-3 dm$^3$ using suspended microbiological rockers. The article proposes and investigates the use of modern innovative equipment – the AF-0.170 self-contained bioreactor with a capacity of 170 dm$^3$, which is adapted to the conditions of small enterprises and enables the performance of key operations in small-scale production of biological preparations. Based on the experimental studies conducted, an operational technology for the cultivation of bacteria in the AF-0.170 bioreactor has been developed. The resulting preparation has a standard titre of viable cells. The total duration of the fermenter operating cycle is 48 hours, with a capacity of 120 dm$^3$. A comparison with production using rocking beds showed a significant reduction in manual labour and electricity costs per unit of output. This creates the conditions for a cost-effective increase in the production volumes of the biorodenticide and a corresponding rise in the level of biologisation of agriculture.

**Keywords:** self contained bioreactor, microbial rodenticide, small-scale production, operational technology.

## Introduction

Currently, the primary biological method for controlling rodents in Ukrainian agriculture is the use of microbiological preparations based on strains of the bacterium *Salmonella enteritidis* var. Issatchenko (hereinafter referred to as S.e.) [1; 2]. Rodents are found on approximately 40% of agricultural land and cause significant economic damage to the crop production sector [1]. The use of chemical pesticides, including rodenticides, is known to have significant negative environmental consequences; consequently, the development of sustainable agriculture worldwide is being driven by the use of biological pesticides [3]. This highlights the relevance of scientific research aimed at improving their production in terms of enhancing economic efficiency and ensuring the quality of biopesticides [3; 4].

Microbiological plant protection products in Ukraine are produced mainly in regional biological laboratories and other small enterprises. Typical technologies are based on manual labour using laboratory equipment and process equipment from other industrial sectors [5]. In particular, the cultivation of S.e. is mainly carried out in glass jars or aluminium containers, which are sterilised together with the culture medium in steam sterilizers. The fermentation stage takes place in an industrial microbiological rocker shaker. The most widely used shaker of KPM-36/90 type, (see Fig. 1), was developed at the Engineering and Technology Institute “Biotechnika” [6]. This technology requires a significant amount of manual labour and makes it difficult to ensure a consistently high-quality product. During inoculation, sampling and packaging, an excessively large opening (more than 8 cm in diameter) must be cut into each container. This creates conditions for airborne contamination.

The standard method of overcoming the shortcomings of shaker technology is the use of fermenters [7]. By developing of new types of fermentation equipment equipped with monitoring and control systems, and exploring promising applications, bioreactors play a key role in addressing global challenges and driving the sustainable advancement of science and technology [8; 9].

However, specialised fermentation equipment for small-scale production of microbiological plant protection products is virtually non-existent in Ukraine or is too expensive. This study proposes the use of modern, innovative equipment – a self-contained bioreactor – for the production of biorodenticides. The self-contained bioreactor AF-0.170 type was developed by the Engineering and Technology Institute ‘Biotechnika’ [10]. The apparatus is adapted to the conditions of Ukraine’s industrial biolaboratories and facilitates the key technological processes involved in the production of

microbiological plant protection products – sterilisation of the culture medium and cultivation of bacterial and fungal preparations [11].

Fig. 1. **KPM 36/90 suspended microbiological shaker**

The aim of this study is to develop a method for culturing S. e. bacteria in a fermenter and to determine its key parameters.

## Materials and methods

Studies of the production processes were carried out using *Salmonella enteritidis* var. Issatchenko strain K-28, which was isolated by the Engineering and Technology Institute 'Biotechnika' and deposited at the Depository of the Institute of Microbiology and Virology of Ukraine under accession number B-7207. Industrial production regulations and technical specifications were developed and approved for the production of the grain biopesticide 'Baktorodencid BT' in volumes of up to 17 tonnes per year. In 2023, Conclusion No. 12.2-18-6/9581 was received from the State Service of Ukraine for Food Safety and Consumer Protection regarding the Technical Specifications (TU U 72.1-00495929-017:2023) for Bactorodencid BT. In 2019, Bactorodencid BT was approved for use in organic farming in accordance with the standard of the International Accredited Certification Bodies for Organic Production and Processing, which is equivalent to the European Union Regulations No. 834/2007 and 889/2008.

The standard technological specifications for the production of a biological rodenticide include the following main stages of the manufacturing process [12; 13]: TP1 – propagation and storage of the initial S.e. bacterial culture; TP2 – preparation of the first-generation inoculum; TP3 – cultivation of the culture in a liquid formulation suitable for use as a commercial product (liquid biological rodenticide) or as a second-generation inoculum for the production of a grain formulation. The subject of this study is the equipment and technology applied at stage TP3. The following main requirements and evaluation criteria were selected:

- suitability for production conditions of small-scale enterprises;
- economic efficiency of the proposed innovation;
- provision of safe and ergonomic working conditions for production personnel.

The AF-0.170 self-contained bioreactor [11; 14] was selected as the technical solution for stage TP3. The fermenter (see Fig. 1, Table 1) is a fermenter-steriliser with mechanical mixing and aeration.

The vessel is equipped with a water jacket and electric heaters. The nutrient medium is mixed by a low-speed, turbine-type mechanical stirrer with variable speed control. Aeration is provided through a bubbler tube that feeds compressed air into the turbine suction zone. Small air bubbles produced by the bubbler are carried outward by the centrifugal force generated by the rotating turbine and dispersed throughout the medium volume. This aeration scheme improves the uniformity of oxygen distribution in the medium [15; 16].

A distinctive feature of the fermenter is its dimensions and the design of the lid with a tilting mechanism (Fig. 1a), which allows the lid to be rotated around its mounting axis using a handle-lever.

The total height of the closed unit is only 1.55 m, which allows a single person to open the cover whilst standing on the floor next to the fermenter without the use of additional lifting devices or ladders [16].

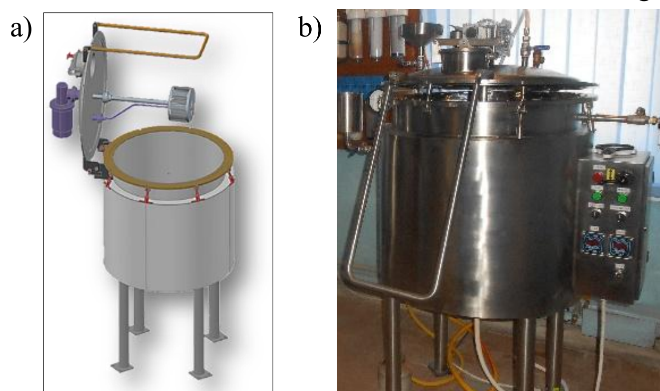

Fig. 1. **Schematic diagram (a) and photograph (b) of the fermenter AF-0.170**

Table 1

**Technical specifications of AF-0.170**

| Indicator Name | Measurement unit | Parameter value |
|---|---|---|
| Total capacity | $dm^3$ | 170 |
| Working capacity | $dm^3$ | 120-130 |
| Stirrer speed | rpm | 55-250 |
| Heater electrical power | kW | 12.5 |
| Rated stirrer power | kW | 0.37 |
| Overall dimensions | mm | 850×750×1550 |
| Mass | kg | 150 |

The fermenter is equipped with a control panel (Fig. 1b) that monitors the stirrer speed and the temperatures in the reactor and the jacket.

The fermenter autonomy is ensured by auxiliary equipment – water and air purification and sterilisation units, as well as a brewing kettle for preparing the nutrient medium concentrate. The operation of both units is based on a multi-stage filtration process. The water purification unit consists of three filters with polypropylene and carbon cartridges and is equipped with a water meter. The air purification unit has polypropylene filters and a flow-through ultraviolet irradiator, and also includes a low-power 280 W compressor and a gas meter.

Experimental studies and technology validation were carried out on operational process equipment during the full-scale production of the biological rodenticide Baktorodencid BT. Microbiological analysis was performed in a specialised department of the institute using standard methods [17]. In particular, the concentration of microbial cells in liquid media was determined in serial dilutions of the bacterial suspension using a Goryaev chamber ($cells \cdot ml^{-1}$) or by counting colony-forming units (CFU) per 1 g using the Koch method. To determine the purity of the bacterial culture, the characteristic features of S.e. var. Isatschenko colonies were observed and smears were prepared and stained using the Gram stain.

## Results and discussion

An operational technology has been developed based on the general technology for the production of microbiological preparations in fermenters [17]; its flowchart is shown in Fig. 3.

The technology for cultivating S.e. in a fermenter at the TP3 stage is implemented using four process units: the AF-0.170 fermenter and auxiliary units: a brewing kettle and water and air purification units. The technology comprises the main technological process operations TP3.1-TP3.4, auxiliary operations AA3.1-AA3.5, as well as control operations K3.1-K3.2. A description of the operations is provided below.

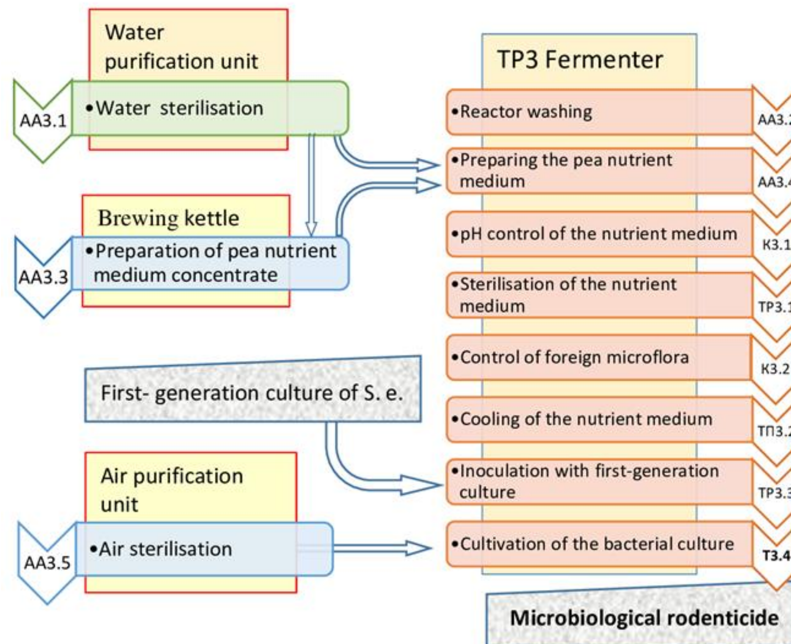


Fig. 3. **Flowchart of the operational technology for the liquid culture of S.e. production process**

AA3.1. Purification and sterilisation of tap water to the required standard by filtration for the preparation of the culture medium.

AA3.2. Preparation of the fermentation apparatus by washing it with running tap water, followed by wiping with cotton wool soaked in a 70% alcohol solution or a 5% hydrogen peroxide solution.

AA3.3. Preparation of a pea nutrient medium concentrate (all quantities are given for a yield of 100 $dm^3$ of final product): 1.0 kg of good-quality pea seeds are washed to remove impurities, placed in a clean enamel container of approximately 2 litres, covered with purified water (2–3 cm above the level of the seeds) and left to stand for 24 hours at room temperature and a further 24 hours at 50 °C in a thermostat. After this, the soaked peas are transferred to a brewing kettle, 20 $dm^3$ of purified water is added, and the mixture is boiled for 30 minutes. The liquid is then filtered through a filter into an intermediate container. The mass ratio of peas to water is 1 to 20.

AA3.4. The reactor of the fermentation apparatus is filled with 80 $dm^3$ of purified water. The volume of water is measured using a meter on the purification unit. The pea nutrient medium concentrate is added to the water in the fermentation apparatus vessel through the open lid. The mixture is homogenised with the lid closed using the fermentation apparatus stirrer at 179 rpm for 15 minutes. The mass ratio of concentrate to water is 1 to 4.

K3.1. Measure pH of the medium, which should be between 7.6 and 7.8. If pH does not meet the required values, adjust it using salts.

TP3.1 To carry out sterilisation, the fermenter jacket is filled with 60 $dm^3$ of filtered tap water, after which the temperature for the inner vessel is set to 120 °C on the temperature controller, and the electric heaters are switched on. Sterilisation takes place at a temperature of 120 °C for 120 minutes.

K3.2. Testing for the presence of foreign microflora is performed by inoculating a sample onto an agar-based nutrient medium and incubating it at 37 °C for 24 hours. The absence of microbial growth on the agar after incubation confirms the sterility of the medium.

TP3.2. Cooling of the nutrient medium from 120 °C to 37 °C takes place via heat exchange with the surrounding air (cooling of the fermenter) and is monitored by a laboratory assistant.

TP3.3. After cooling the medium, the fermenter is inoculated with a first-generation culture of *Salmonella enteritidis* var. Issatchenko strain K-28 (S. e.). The volume of the inoculum is (1.51.8) $dm^3$ (1.5% of the volume of the nutrient medium) with a titre of viable microbial cells of $4\times10^{10}$ $CFU\cdot cm^{-3}$. If the titre is lower, the volume is increased accordingly. The duration of bacterial culture growth is 24 hours. During this time, a constant temperature of 37 °C and an aeration rate of 0.3 $dm^3\cdot min^{-1}$ per

$dm^3$ of nutrient medium are maintained. The required mass transfer is ensured by a stirrer rotating at a speed of 179 rpm.

The post-fermentation stage involves transferring the culture fluid to a buffer tank under sterile conditions, monitoring its titre, and its subsequent use – either as a biological rodenticide or as a second-generation starter culture in the production of a grain-based formulation.

The fermentation process lasts 24 hours at a temperature of 37 °C, with an aeration rate of 0.3 $dm^3 \cdot min^{-1}$ per $dm^3$ and a stirrer speed of 179 rpm. The resulting S. e. culture has a titre of $1.0 \times 10^9$ $CFU \cdot cm^{-3}$, which meets the standard. Taking into account a two-shift operating regime, the total duration of the fermenter cycle will be 2 days. The maximum productivity per cycle is (120-130) $cm^3$ of the preparation, yielding a minimum daily productivity of 60 $cm^3 \cdot day^{-1}$. The technology description also provides the values of the main technological parameters, which were determined based on the results of experimental studies in the production of the S. e. bacterial culture.

The economic efficiency of using the AF00.170 fermenter and the developed technology in the production of a biological rodenticide, compared with the KPM 30/90 shaker, was assessed on the basis of Production Costs and Payback Period. The daily output of the shaker is 54 $dm^3$, whilst that of the fermenter is 60 $dm^3$. The energy consumption for sterilisation and fermentation in AF-0.170 was determined experimentally and is shown in Table 2.

Table 2

**Energy consumption of the AF-0.170 fermenter during fermentation of S.e.**

| Process | Parameter | Measurement unit | Parameter value |
|---|---|---|---|
| Mixing | Stirrer speed | rpm | 179 |
| | Stirrer operating time | hours | 24 |
| | Stirrer power consumption | kW | 0.37 |
| | Energy consumption | kWh | 8.9 |
| Aeration | Aeration air flow rate | $m^3 \cdot min^{-1}$ | 36 |
| | Aeration time | hours | 24 |
| | Compressor power consumption | kW | 0.28 |
| | Energy consumption | kWh | 6.7 |
| Thermoregulation | Internal container temperature: sterilisation or fermentation | °C | 120/37 |
| | Heater electrical power | kW | 12.5 |
| | Actual operating time of heaters | hours | 4.2 |
| | Energy consumption | kWh | 52.5 |
| | **Total energy consumption for fermentation** | **kWh** | **68.1** |

The specific energy consumption for producing 1 $dm^3$ of the preparation is 0.57 $kWh \cdot dm^{-3}$. The specific energy consumption for sterilising culture flasks in a 45 kW steam steriliser followed by fermentation in the shaker is 1.15 $kWh \cdot dm^{-3}$ [11]. Thus, energy consumption in the fermenter is reduced by a factor of 2. According to current tariffs and prices in Ukraine, this will lead to a reduction in the production cost of the biorodenticid by 1.33 $USD \cdot dm^{-3}$, which amounts to approximately 16%. Given that the annual output of the AF-0.170 fermenter is at least 2.400 $dm^3$, its payback period is calculated at 2.2 years, which demonstrates the economic efficiency of the innovation.

## Conclusions

1. An innovative technical solution for the production of a liquid biological rodenticide has been validated, enabling the replacement of a set of equipment comprising small-scale culture vessels, a steam steriliser and a suspended microbiological shaker with a self-contained fermenter with a capacity of 170 $dm^3$, which meets the requirements for small-scale production of biological plant protection products. Decreasing the number and upgrading the production equipment reduced the required production floor space by a factor of three and enabled the transition to a modern production process.

2. A new operational technology has been developed for the cultivation of *Salmonella enteritidis* var. Issatchenko in a self-contained fermenter, which has ensured the production of a preparation meeting regulatory quality standards at a rate of 60 dm$^3$ per day. This has led to a significant improvement in working conditions for the staff through mechanisation and automation of the production process, and has also ensured higher quality standards for the biopesticide by reducing the risk of contamination by foreign microflora.
3. Due to the reduction in energy costs in production, the production cost price of the biorodenticid is expected to decrease by 16%. With an annual productivity of the fermenter of 2.400 dm$^3$, its payback period is calculated at 2.2 years, which determines the economic efficiency of the innovation.

## Author contributions

Conceptualization, I.B.; O.B. and A.A.; methodology, N.P. and A.R.; software, M.P.; validation, A.A. and I.B; formal analysis, OV.B and R.V..; investigation, A.A., A.R., I.P. and R.P.; data curation, A.A., A.R. an N.P.; writing – original draft preparation, A.R.; writing – review and editing, A.A. and O.B.; visualization, M.P., N.P.; project administration, A.A.; funding acquisition, A.R. All authors have read and agreed to the published version of the manuscript.

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