RESEARCH OF THE CLEANING SYSTEM FOR THIN-WALLED FERMENTER, USED IN THE MANUFACTURING OF MICROBIAL PLANT PROTECTION PRODUCTS
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ДОСЛІДЖЕННЯ СИСТЕМ ОЧИЩЕННЯ ТОНКОСТІННОГО ФЕРМЕНТЕРУ ПРИ ВИРОБНИЦТВІ МІКРОБІОЛОГІЧНИХ ЗАСОБІВ ЗАХИСТУ РОСЛИН

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DOI: https://doi.org/10.35633/inmateh-66-12

Keywords: *thin-walled fermenter, cleaning, pasteurisation, steriliser, heated water.*

ABSTRACT

The peculiarities of microbial plant protection products from pests and diseases allow the use of thin-walled fermenters in their production, in which there is no sterilisation under high pressure. The article is devoted to the development of the cleaning in place system for such fermenters. Experimental studies have been carried out to ensure a pasteurising effect when treating the fermenter with heated sterile water and a nutrient medium concentrate obtained from the sterilizer. Its parameters: its volume, temperature, the time delay have been determined. There is proposed a hardware diagram of the fermentation plant, consisting of an industrial steriliser and a thin-walled fermenter. The cleaning procedure is combined with the main technological process and does not require additional equipment. The production tests have confirmed the cleaning efficiency.

АНОТАЦІЯ

Особливості мікробіологічних засобів захисту рослин від шкідників і хвороб дозволяють при їх виробництві використовувати тонкостінні ферментери, в яких відсутня стерилізація під високим тиском. Стаття присвячена розробленню системи очищення на місці для таких ферментерів. Проведено експериментальні дослідження із забезпечення ефекту пастеризації при обробленні ферментеру нагрітою стерильною водою й концентратом поживного середовища, що пройшло стерилізацію. Відзначені її параметри: об'єм, температура, час витримки. Запропоновано апаратурну схему ферментаційної установки, що складається з промислового стерилізатору і тонкостінного ферментеру. Процедура очищення відбувається сумісно з основним технологічним процесом й не потребує додаткового устаткування. Ефективність очищення підтверджено промисловими випробуваннями.

INTRODUCTION

Ecologisation of agriculture as a strategic direction of sustainable development of environmental activities provides for the widespread use of microbial plant protection products from pests and diseases (hereinafter biopesticides) *(Liu et al., 2021; Arshad, 2018; Van Oosten, et al., 2017; Nicolopoulou-Stamati et al., 2016)*. It has historically occurred in Ukraine that their production, despite the presence of the microbiological industry, was carried out mainly in biological laboratories - small enterprises that were part of the agricultural system. This structure is still in effect and is the most promising at the deficiency of investments *(Krutyakova, 2019).*

Microbiological production, as a branch of industry, has significant and sufficient scientific support *(Panda, 2011; Köhler et al., 2015; Yaroshevsky et al., 2020*). Its main processes - sterilisation and fermentation - have many hardware and technological implementations (*Lukanin, 2020).* Yet, as practice shows, most of them have not found application in biological laboratories due to the unacceptable cost of classic high-pressure fermenters, significant costs of thermal sterilisation and, most importantly, the inexpediency of creating microbiological production facilities with full infrastructure at small enterprises.

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It is known that a necessary condition for the functioning of the fermentation complexes is the presence of two systems (*Lukanin, 2020; Goode et al., 2013; Pettigrew et al., 2015)*. The equipment cleaning by CIP-system (Cleaning-in-Place) for ensuring the required level of equipment cleanliness. The processing is performed in several stages: rinsing with water, rinsing with a detergent reagent, rinsing to neutralise the reagent. The equipment sterilisation by SIP-system (Sterilisation-in-Place) without its preliminary dismantling. Typically, saturated steam is used for sterilisation at a temperature of 120°C and a pressure of 2 bars for about 60–70 minutes. The developed variety of the sterilisation modes testifies mainly to the fact that these modes have a practical basis and are not based on the theoretical foundations of the present-day thermal sterilisation. The existing approach to the assessment of the efficiency of the sterilisation regimes does not allow determination of the efficiency that is really necessary for guaranteed inactivation of the extraneous microflora (*Lukanin, 2020)*.

Usually, these systems are implemented by means of additional equipment as part of a complex with the high-pressure fermenters, being created for the food and pharmaceutical industries. Analysis of the production and use of biopesticides showed that in most cases of conditionally aseptic production is sufficient (*Kotlyarov and Sedinina, 2014; Krutyakova et al., 2017)*. This is primarily due to the marketable form of the preparations and their purpose when the product is a culture liquid from fermenters with a short shelf life, which is used to treat plants or the soil. Therefore, the so-called thin-walled fermentation devices have been developed specifically for the production of biopesticides at the Engineering and Technological Institute "Biotekhnika" (*Starchevsky et al, 2007; Krutyakova et al, 2019)*.

In thin-walled fermenters the metal consumption is significantly reduced in contrast to the industrial fermenters due to their manufacture from thin-sheet corrosion-resistant steel (δ = 0.8...1.5 mm). The pressure inside the fermenter is almost equal to the atmospheric pressure, which automatically eliminates the requirements of the government regulations for pressure vessels.

The disadvantages of the thin-walled reactors were problems with sterilisation of the equipment, which necessitated application of special methods. Treatment with disinfectant solutions, heating of the metal surfaces to high temperatures *(Palabiyik et al., 2015; Wilson et al., 2015)*, and other methods were used for such fermenters sterilization. The purpose of this work was to develop a cleaning system with the sterilisation elements for a modern thin-walled fermenter *(Bespalov and Khodorchuk, 2017)*.

MATERIALS AND METHODS

Long-term operation of the fermentation plants suggested one more method of cleaning and sterilisation "in place" without the use of additional tools or devices. The known methods of pasteurisation of the food products were used *(Bredikhin, 2021)*, which ensure the death of vegetative forms of microorganisms. The efficiency of pasteurization depends on two parameters: the temperature to which the product is heated, and its exposure at this temperature.

Numerous data for the dairy products indicate that at temperatures above 85°C, an exposure of 10 minutes is sufficient. These values were used as a reference in the design of the cleaning system. The experimental equipment (See Fig. 1), on which the research was performed, is a part of the pilot fermentation plant *(Bespalov and Khodorchuk, 2017)* at the Engineering and Technological Institute "Biotekhnika" (See Fig. 2).

The thin-walled fermenter with a 115 litres capacity was made of stainless steel with a thickness of 1...2 mm. It consists of vessel *3*, cover *2*, air jacket *4* through which the ambient air was pumped by fan *5* for cooling. Sterile water was supplied through valve *V1*. Agitation was made by sterile air supplying under pressure through valve *V3* into the liquid volume through the nozzle *1*.

An industrial autoclave VK-75 was used as a sterilizer. The sterilizer had the sterilization chamber *6* with water jacket *8* between which valve V9 is installed. The sterile water is supplied to the chamber through valve *V5*.

Concentrated nutrient medium (CNM) making procedure involved CNM components addition through open lid *7* into water volume, contained in chamber *6*, further homogenization of mixture by pressured air, which was supplied through valve *V8*, and thermal sterilization at 2 bars according to the standard method. The heated steam-water mixture, after reaching the set temperature or sterile CNM with the same temperature, is fed from the sterilizer to the fermenter through a flexible corrugated stainless-steel pipe *10,* with detachable connections.

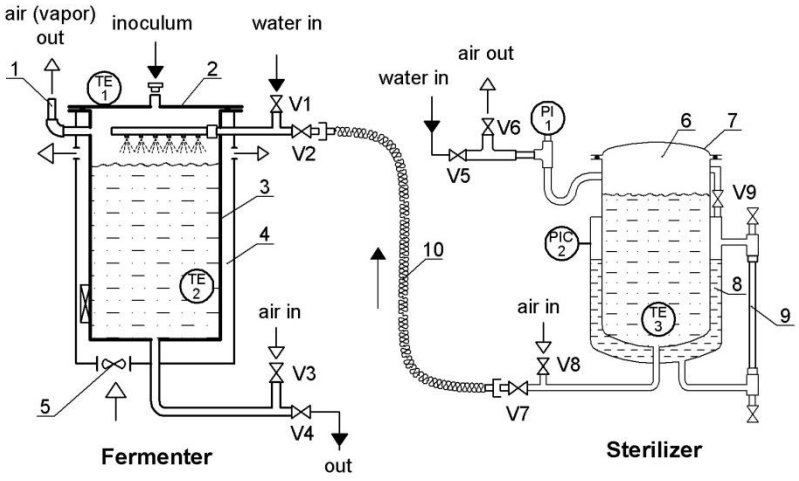

Fig. 1 - The hardware diagram of the experimental fermentation plant

Fig. 2 - The pilot fermentation plant

The bypass is carried out under the action of the sterilisation pressure in chamber *6* by opening valves V7, V2, valve V9 being closed to preserve water in jacket *8*. The measurement of the parameters in the experiment was conducted by a set of instruments with an accuracy class of 0.5, including:

t_C – temperature of the outer surface of cover *2* by the TE in sensor *1*;

t_V – temperature inside the fermenter vessel by TE in sensor *2*;

t_S – the temperature of the liquid in sterilizer chamber *6* by the TE in sensor *3*;

t_a – the ambient air temperature in the room (sensor not shown).

Temperature t_S was controlled using the electrocontact manometer PIG / 2. Statistical processing of the measurements was performed using the MS Excel spreadsheet according to the standard method (Dally, 2008). During data processing there were determined the arithmetic mean values of the measured values, the standard deviation; the relative and absolute measurement errors were calculated for a 95% confidence level.

Cleaning of the fermenter and pipelines was carried out with tap water, which was heated in the sterilizer to an average temperature of t_s = 127.0°C, and then, under surplus pressure in the sterilizer, it was released into the fermenter, the fermenter communicating with the atmosphere through outlet pipe 1 (Fig. 1). The average air temperature in the room was t_a = 20.6°C. In the first experiment there were taken 10 dm^3 of water in 4 replicates, which provided the maximum average temperature in the fermenter, equal t_{V1} = 91.8°C. At atmospheric pressure this value cannot exceed 100°C, and the task of the research was maximum approximation to this value. In the second experiment, in 6 replicates, the volume of water was increased to 16 dm^3.

In statistical processing *(Nicolopoulou-Stamati et al., 2016)* there was used the criterion of Student's test 2.57 for the significance level of 0.05. When the temperatures were decreasing, ε increased, but this did not change the main result with value t_{V1} = 98.3°C, which was considered sufficient.

RESULTS

The graphs of the arithmetic mean temperatures of the vapour-air medium in the vessel - t_V and the outer surface of the cover - t_C are shown in Fig. 3. The measurements were made at time points 0; 1; 5; 10; 25 minutes. The maximum of heating was reached in 1 minute, and it amounted to:

t_{V1} = 98.3±1.8° C, MSD =1.84 %

t_{C1} = 95.0±2.5° C, MSD =2.63 %,

where MSD is the mean-square deviation.

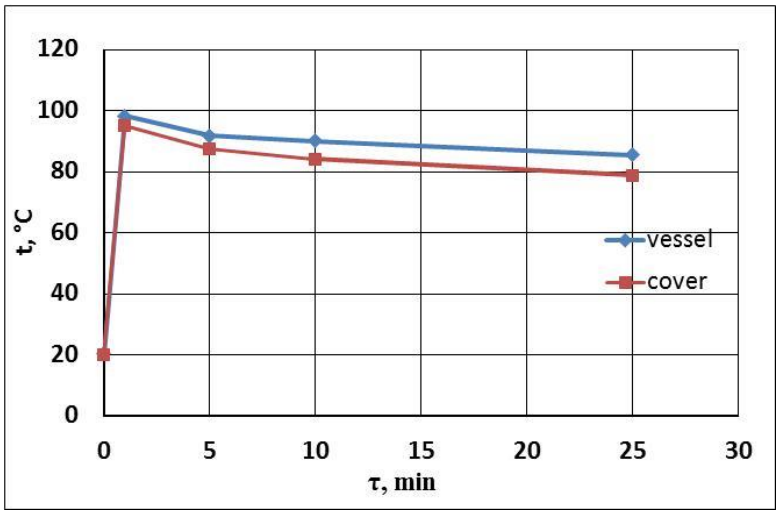

Fig. 3 - The fermenter temperature during pasteurization

Logical analysis shows that the temperature of the internal surfaces of the fermenter for our design must satisfy the ratio:

$$t_c < t_f < t_v \quad (1)$$

where: t_f – average temperature of the inner surface of the fermenter.

The obtained graphs (Fig. 3) show that the temperature t_f > 85°C is maintained for 10 minutes, i.e., the pasteurization mode inside the vessel is ensured. MSD increased when the temperatures were decreasing. However, this did not change the main result with value t_{V1} = 98.3°C, which was considered sufficient.

The water with a temperature of about 85°C is discharged by gravity from the fermenter after the end of the holding time, while opening its outlet pipe with valve V4. Cleaning the bypass line between the sterilizer and the fermenter (outlet and inlet nozzles, valves V2, V7, flexible pipe 10) takes place by boiling water within the first minute at a temperature of at least 100°C. The amount of water for cleaning was selected in accordance with the technological regulations for this fermentation plant. The regulations include the following ratios:

$$\upsilon_u = \upsilon_s + \upsilon_w + \upsilon_i, \quad (2)$$

$\upsilon_s : \upsilon_w$ = 1:4, υ_i = (3-5 %) υ_u,

where:

υ_u = 84 dm^3 – the useful volume of the fermenter;

υ_s = 16 dm^3 – the volume of the CNM;

υ_w = 64 dm^3 – the volume of sterile water;

υ_i = 4 dm^3 – the inoculum volume.

After cleaning is completed, the next technological stage is performed - nutrient medium making in the fermenter. In the sterilizer a concentrated nutrient medium υ_s = 16 dm^3 is prepared, being sterilised at t_s = 127.0°C, and then supplied into the fermenter, with its thermal effect proceeding similar to the cleaning process by heated water, and accompanied by the same pasteurizing effect. After cooling the CNM, cold sterile water is added to the fermenter in ratio (1). The cyclogram of the fermenter cleaning processes in the form of temperature changes in the tank t_V at different stages of the technological process is shown in Fig. 4. Sterile water is poured into the sterilizer with volume υ_w; at the moment τ_0 = 0, heating is turned on, which ends at t_s = 127.0°C. The sterilizer turns off, valves V2, V7 are opening, the steam-water mixture flows into the fermenter, heating its volume at the moment τ_1 to temperature t_{V1} = 98.3°C. Then the fermenter cools down to temperature t_v = 85°C. Holding at these temperatures determines the high-temperature pasteurisation on the inner surfaces of the fermenter. After that valve V4 opens, and hot water is released into the sewer, cleaning the outlet pipe with a tap.

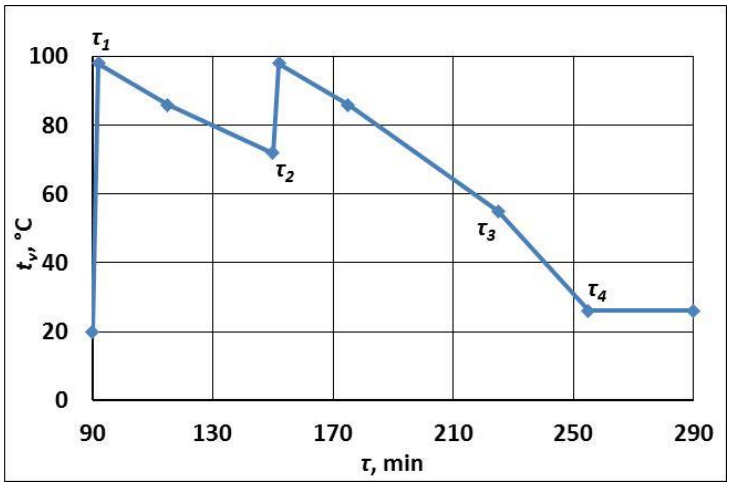

Fig. 4 - Cyclogram of the fermenter cleaning processes

The subsequent stages of cleaning with a pasteurizing effect are already associated with the implementation of the main technological process. After freeing the sterilizer from water at the moment of time τ_1, a concentrated nutrient medium is prepared in it, the sterilisation of which ends at the moment of time τ_2. Next, the bypass of the CNM opens into the fermenter.

The second stage of heating and pasteurization of the fermenter takes place, as it was described earlier. This process ends in 10 minutes, and air cooling of the fermenter is turned on by fan *5* in the jacket. The cleaning process is complete. When temperature t_{v3} drops to 50-55°C at the moment τ_3, sterile water with a volume of υ_w at 20-25°C is supplied to the fermenter to dilute the CNM to the required concentration of the nutrient medium. By choosing ratios υ_w, υ_s, t_{v3} it is possible to regulate the final temperature of the nutrient medium at the end of the dilution at moment τ_4, usually 25...28°C. This indicates the end of the pre-fermentation stage. Inoculation with the seed culture is carried out, and then - the cultivation process starts. The research was carried out under the conditions of real production of biopesticides.

The cyclogram presents maximum values of the stage durations, which were recorded at a particular fermentation plant. For a fermenter with a different volume, it is necessary to select volumes υ_s; this will change the duration of the operations. The developed method provided double heating of the inner surfaces of the fermenter to temperatures that guarantee the implementation of the pasteurizing effect. The system for cleaning the fermentation plant from a 75-litre steriliser and a 115-litre fermenter made it possible to implement the cleaning and pre-fermentation stage in 4.0 ... 4.5 hours, that is, one steriliser can serve two fermenters during a working shift. This later determined the optimal composition of the unit *(Krutyakova et al., 2019).*

The final assessment of the cleaning system was made on the basis of the results of the pilot production of two complex biopesticides "Biohybervit BT" TU U 72.1-00495929-026: 2020 and "Vitastim BT" TU U 72.1-00495929-025: 2020, based on two strains of bacteria *Pseudomonas fluoresens* and two strains of the antagonist fungus *Trichoderma lignorum*. The titre of biopesticides was within (1-4)·10^9 CFU cm^{-3} (colony-forming units). The amount of the extraneous microflora was less than the value of 0.3%, established by the Technical Specifications.

CONCLUSIONS

A CIP-system for a fermentation plant has been developed, which consists of an industrial steriliser and a thin-walled reactor. Cleaning from contamination is combined with a pasteurizing effect, which ensures the death of vegetative forms of microorganisms on the inner surface of the equipment.

Cleaning is performed by water with the initial temperature of 98.3°C without the use of additional devices. The pasteurizing effect is achieved by heating the inner surfaces twice to a temperature of more than 85°C with holding for 10 minutes.

The cleaning procedure is combined with the main technological process and does not require additional equipment. Testing of the system in the production of biopesticides has confirmed its efficiency. The level of contamination by extraneous microflora did not exceed the established standard value 0.3%.

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