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THE EVALUATION OF MICROBIAL AIR CONTAMINATION IN MICROBIAL PESTICIDE MANUFACTURING IN BIOREACTORS

YAROSHEVSKY V.*, OSIPENKO Tatyana**, YAROSHEVSKA Julia***
Engineering and Technological Institute "Biotekhnika" of the National Academy of Agrarian Sciences of Ukraine
**https://orcid.org/0000-0002-1905-2092,*
***https://orcid.org/0000-0001-6419-9520,*
****https://orcid.org/0009-0003-6587-2678,*
e-mail: wladscience@gmail.com

Summary. The paper is dedicated to actual scientific and practical problem solutions for air treatment in microbial pesticide manufacturing. Experimental results of air filtration in series-link filters with 5 μm – 1 μm rating polypropylene cartridges are shown. Obtained microbial contamination level of filtered air 3.4 CFU·m^{-3} was low and air quality seemed to be sufficient for fermentation broth aeration in the bioreactor. The reasons for such results and prospects of filtration system simplification for pilot-scale microbial pesticide manufacturing are discussed.

Keywords: *microbial pesticides, microbial air contamination, pollution, filtration*

Introduction. Microbiological air pollution is a very serious problem for fermentation technology in microbiological, pharmaceutical, food, and chemical industries. Microbial pesticide production technology is not an exception. As most biopesticides are based on aerobic fungi and bacteria cultures [1, 2], so microbial growth needs to be provided by all the time air supply [3]. Thus, ambient air cleaning and sterilization are obligatory procedures in the technological process [4].

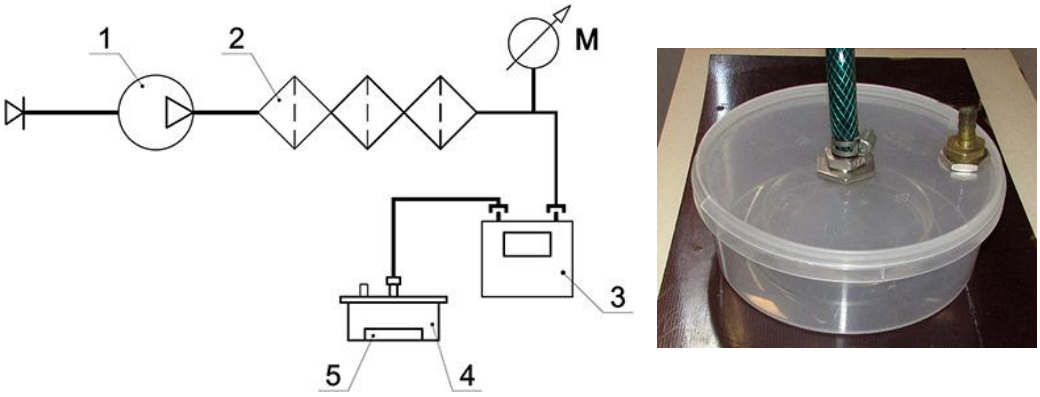
The standard procedure of air decontamination in fermentation technology expects filtration through 0.1-0.4 μm rating microbial filters. However, filtration consumables (membranes) are very expensive. It is believed that filtration material should be polluted after one fermentation cycle. Therefore, it must be changed after each cycle. Thus, air cleaning operation costs turn out to be significant.

The situation determines searching the ways for air treatment cost reduction. One of the solutions to this problem was proposed in Engineering and Technological Institute "Biotekhnika" of the National Academy of Agrarian Sciences of Ukraine (ETI “Biotekhnika” NAAS). It essence reduces to air filtration by 1 μm rating filters in combination with additional disinfection by UV or ozonation. Such filters and their consumables (cartridges) cost far less than microbial membrane ones. In another hand, we cannot predict the air purification efficiency needed for further UV or ozone treatment system calculation and equipment. Microbial contamination level of filtered air should be evaluated experimentally for this purpose.

Materials and Methods. The main goal of this work was the experimental evaluation of microbial contamination of air passed through the simple filtration system, equipped with polypropylene (PP) 5 μm – 1 μm rating cartridges.

The Air Filtration Module (AFM) of Fermentation Unit FU-500 [5] was used for the experiments (Fig. 1). FU-500 is the technological line for pilot-scale microbial pesticides production, which is equipped with two 315 dm^3 bioreactors [6]. The AFM aimed to filtration of ambient air and control of growth medium aeration rate in bioreactors. It consists of the air compressor, filter system, gas meter, and manometer. The filter system contains three series-link filters with 5 μm – 1 μm rating polypropylene (PP) cartridges. Experimental facilities completed by the air sampler of simple design created especially for the experiments (Fig. 2a). The sampler consists of the circle container of 1 dm3 volume with two nipples on the top required for air in and out (Fig. 2b).

Fig. 1. Air Filtration Module of Fermentation Unit FU-500
(without installed PP cartridges)



a) b)

1 – air compressor, 2 – filter system (5 μm – 1 μm), 3 – gas meter, 4 – air sampler, 5 – Petri dish, M – manometer.

Fig. 2. Experimental facilities scheme (a) and air sampler general view (b)

The experimental procedure was equal to methods used for compressed air pollution evaluation [7]. Petri dish with Gromyko agar media (nutrient agar : Sabouraud agar = 1:1) put into the sampler. After that air jet was sprayed into the media for the determined time (10 min). Then Petri dish took away from the sampler and incubated for 72 hours at 27 ºC. The microbial pollution of agar media was evaluated by the Koch method [8]. The result was the total viable count in CFU·m^{-3} of the air volume (filtered or unfiltered) passed through the Petri dish during time intervals [7, 8]. The experiments were made in triplicate for filtrated and unfiltered air.

Koch sedimentation method during 30 min was used for evaluation of microbial contamination of air in the laboratory, from which air was injected by compressor [8, 9].

Results and Discussion. The experiments were carried out in the summer period. Ambient air parameters were: temperature t = 27.5 ºC, atmospheric pressure Pa = 100.53 kPa, humidity φ = 52 %. Average airflow and surplus pressure in the controls were Qc = 80.9 dm3·min–1, Pc = 3.92 kPa, and in the tests Qe = 96.3 dm3·min–1, Pc = 5.88 kPa. Air was sampled after filters in controls and the tests both, but in the controls filter tanks were empty, i. e. without PP cartridges. The microbial contamination in experiments was evaluated for 747-949 dm3 of air passed through the sampler (table 1).

Table 1. Microbial air contamination

No	Experiments	Air volume passed through the sampler, dm^3	Microbial air contamination, $CFU \cdot m^{-3}$			Average total viable count, $CFU \cdot m^{-3}$
			bacterial	fungal	total	
1	Control 1	839.4	3.6	4.8	8.3	6.3
2	Control 2	747.1	2.7	4.0	6.7	
3	Control 3	784.3	2.5	1.3	3.8	
4	Test 1	948.9	3.6	1.2	4.8	3.4
5	Test 2	933.6	3.2	0	3.2	
6	Test 3	869.0	0	2.3	2.3	
Controls – unfiltered air, Tests – filtered air						

As one can see from the table microbial contamination level of air filtered through PP cartridges was very low – 3.4 $CFU \cdot m^{-3}$. In our opinion, such a purification level seemed to be enough for air treatment used in the aeration of growth medium in bioreactors. Thus there is no need for additional air treatment by UV or ozonation.

In despite of two times higher than pollution level of filtered air, the contamination of unfiltered air was very low either – 6.3 $CFU \cdot m^{-3}$. Taking into account the fact of air suction from the lab room, such microbial pollution of ambient air in the lab corresponds to B Grade by Guidelines [9]. However, there was no pretreatment of air and lab surfaces by chemicals or UV before the experiments. The lab room advisedly was not disinfected for the filtration system capabilities evaluation in severe conditions. Thus ambient air microbial contamination should be much higher, which was truly confirmed by sedimentation control. The average total viable count for sedimentation was $3 \cdot 102$ $CFU \cdot m^{-3}$. Thus microbial pollution level of the same air (without filtration) in the sampler was 50 times lower than sedimented on the Petri dish. Nevertheless, it was not an experimental error, as the test was repeated for another media (Sabouraud agar) and equal results were obtained.

In our opinion, such a significant reduction of microbial air pollution can be connected with experimental procedures and filter structure. Before a series of control experiments filter tanks' inner surfaces were treated with 96 % ethyl solution. The ethyl vaporization exactly was the crucial effect for air

decontamination in the filter tank. This effect was prolonged for all experiment series due to filter structure, as long filter tanks (20 cm) were used. The inlet and outlet both are located at the filter top. Airflow inleted the filter (without PP cartridge) and changed its direction to the outlet before the contact of all tank surfaces. Therefore some inner tank aria (presumably on the bottom) saved ethyl solution during the experimental series. That was confirmed by the ethyl smell arising while tank demounting for PP cartridges installation before tests. At the same time, there was no ethyl smell estimated in the air outlet from the sampler, which indicates that ethyl vapor concentration in the outlet airflow was not high.

The same situation took place in the tests. Air was filtered in PP cartridges and then additionally disinfected by ethyl vaporization. It seems to be the reason for such a significant reduction of microbial air contamination in comparison with one ambient air.

Thus such low microbial air contamination level was obtained due to the complex effect of filtration and ethyl vaporization. As this effect was not predicted, it should be examined in more detail, especially in the case of growth inhibition of target microorganisms in aerated fermentation broth.

Conclusions. Despite the necessity of further research, the experiments showed a high level of air purification by using simple PP cartridges with a higher rating (≥ 1 μm) than required for microbiological filters (< 0.5 μm). We suppose that such air filtration level with a total viable count less than 5 $CFU \cdot m^{-3}$ should be sufficient for aeration of growth medium in bioreactors for microbial pesticides manufacture.

The required level of air microbial pollution for the manufacture of sterile medicinal products for humans is < 1 $CFU \cdot m^{-3}$ – A Grade by Guidelines [9]. However, microbial pesticides are intended for plants and soil treatment but not for humans. Thus such purity is not justified from the point of solution safety for plants.

In another hand, the biopesticides solution's microbial contamination absence is the guarantee of their prolonged storage. However microbial pesticides in Ukraine and Moldova are manufactured basically in biolaboratories in limited volume made to order. The time between biopesticides' manufacture and use, as usual, is no longer than two weeks. Thus, there is no long-term product storage requiring a high aseptic level of growth mediums as well as air and other substrates consequently [6].

According to these reasons, we proposed the approach called "conditionally aseptic" for small-scale and pilot-scale microbial pesticide manufacturing in our previous work [5]. Toward air processing for growth media aeration in bioreactors we suppose that such "not full" sterilization will be quite enough for a biopesticide culture medium of proper quality obtaining. Additionally, such simplification of air quality requirements leads to significant air treatment cost reduction by using filters with simple and cheap PP cartridges.

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