Kaliuzhnaia O.S.,
National University of Pharmacy
E-mail: kalyuzhnayao.s@gmail.com
Kaliuzhnyi O.B.,
Kharkiv Petro Vasylenko National Technical University of Agriculture
mail: albokal@ukr.net
Soloviova A.V.
National University of Pharmacy

INVESTIGATION OF THE USE OF FLUOROPLASTIC FILTER ELEMENTS IN THE PRODUCTION OF A PROMISING ANTIBIOTIC SUBSTANCE PYOCYANIN

УДК 615.014 DOI 10.37700/ts.2020.21.26-32

*O.S. Kaliuzhnaia, O.B. Kaliuzhnyi, A.V. Soloviova "**Investigation of the use of fluoroplastic filter elements in the production of a promising antibiotic substance pyocyanin"***

The paper shows the prospects of further research on the use of porous filters based on fluoroplastic in the production of antibiotic subtancies on the example of pyocyanin.

The analysis of research has revealed the urgency of the problem of antibiotic resistance of infectious agents, in this regard, there is a need to develop new types of antibiotics or improve existing ones.

Today, the main method of obtaining new antibiotics is biosynthesis, which is carried out by fermentation of the corresponding microorganism, and the final stage of any biotechnological production is the isolation of the target product from the nutrient medium and its purification. The initial stage of product isolation is filtration using filters of various designs. Among the large number of filter designs and filter elements of various compositions offered on the market, we focused on fluoroplastic filter elements.

We determined the filtration efficiency of the culture fluid P. aeruginosa - a producer of the antibiotic substance pyocyanin through fluoroplastic filters, studied the permeability of the filter element and the influence of the filter element thickness on the filtration process. Separation of culture fluid of P. aeruginosa from biomass through fluoroplastic filters showed filtration efficiency. Carrying out series of filtrations using the same filter showed preservation of permeability of a filter element. The study of the influence of the thickness of the filter element on the filtration process showed an increase in the efficiency of filtration with increasing thickness.

***Keywords**: fluoroplastic filter elements, production of an antibiotic substance, pyocyanin*

*Калюжная О.С., Калюжный А.Б., Соловьева А.В. **«Исследование возможности использования фторопластовых фильтрующих элементов в производстве перспективной антибиотической субтанции пиоцианина»***

В работе показана перспективность дальнейших исследований по использованию пористых фильтров на основе фторопласта в производстве антибиотических субтанций на примере пиоцианина.

Анализ исследований выявил актуальность проблемы антибиотикорезистентности инфекционных агентов, в связи с этим возникает необходимость разработки новых видов антибиотиков или совершенствования существующих.

Сегодня основным методом получения новых антибиотиков является биосинтез, который осуществляется ферментацией соответствующего микроорганизма, а завершающий этап любого биотехнологического производства - выделение целевого продукта из питательной среды и его очистка. Начальным этапом выделения продукта выступает фильтрация. Среди большого количества конструкций фильтров и фильтровальных элементов различных композиций, предлагаемых на рынке, мы остановились на фторопластовых фильтрующих элементах.

Нами определена эффективность фильтрации культуральной жидкости P. aeruginosa - продуцента антибиотического вещества пиоцианина через фторопластовые фильтры, изучена проницаемость фильтроэлемента и влияние толщины фильтроэлемента на процесс фильтрации. Отделение культуральной жидкости P. aeruginosa от биомассы через фторопластовые фильтры показало эффективность фильтрации. Проведение серий процессов фильтраций сквозь один и тот же фильтр показало сохранение проницаемости фильтроэлемента. Изучение влияния толщины фильтроэлемента на процесс фильтрации показало увеличение эффективности фильтрации при увеличении его толщины.

***Ключевые слова:** фторопластовые фильтрующие элементы, производство антибиотических веществ, пиоцианин*

*Калюжная О.С., Калюжний О.Б., Соловьйова А.В. **«Дослідження можливості використання фторопластових фільтруючих елементів у виробництві перспективної антибіотичної субтанції піоціаніну»***

У роботі показана перспективність подальших досліджень щодо використання пористих фільтрів на основі фторопласта у виробництві антибіотичних речовин.

Аналіз досліджень виявив актуальність проблеми антибіотикостійкості інфекційних агентів, у зв'язку з цим виникає необхідність розробки нових видів антибіотиків або вдосконалення існуючих.

Сьогодні основним методом отримання нових антибіотиків є біосинтез, який здійснюється ферментацією відповідного мікроорганізму, а завершальним етапом будь-якого біотехнологічного виробництва є виділення цільового продукту з живильного середовища та його очищення. Початковим етапом виділення продукту є фільтрація за допомогою фільтрів різної конструкції. Серед великої кількості конструкцій фільтрів та фільтрувальних елементів різних композицій, що пропонуються на ринку, ми зупинились на фторопластових фільтруючих елементах.

Нами визначено ефективність фільтрації культуральної рідини P. aeruginosa - продуцента антибіотичної речовини піоціаніну через фторопластові фільтри, вивчено проникність фільтроелементу та вплив товщини фільтроелементу на процес фільтрації. Відділення культуральної рідини P. aeruginosa від біомаси крізь фторопластові фільтри показало ефективність фільтрації. Проведення серій фільтрацій скрізь один і той же самий фільтр показало збереження проникності фільтроелементу. Вивчення впливу товщини фільтроелементу на процес фільтрації показало збільшення ефективності фільтрації при збільшенні його товщини.

Ключові слова: *фторопластові фільтруючі елементи, виробництво антибіотичних речовин, піоціанін*

Introduction

Increasingly widespread use of antibiotics, especially the misuse, has led to the rapid appearance of antibiotic resistant strains today. This problem is associated with failures in the treatment of various infections. Antibiotic resistance leads to increased mortality, a significant increase in treatment costs. The therapeutic efficacy of antibiotics such as penicillin, erythromycin, syntomycin and others has declined sharply, so the question of finding new antibiotics that have not been widely used before has become acute [1]. Promising antibiotic substance is a phenazine compound - pyocyanin, produced by the bacterium *Pseudomonas aeruginosa* [2].

The main method of obtaining new antibiotics is biosynthesis, which is carried out by fermentation of the corresponding microorganism, and the final stage of any biotechnological production is the isolation of the target product from the culture medium and its purification. The initial stage of product isolation is filtration using filters of different designs [3].

It is known that an important component of the filter is a filter material, which carries out and has a significant impact on the quality of filtration and the performance of filter devices.

Among the large number of filter designs and filter elements of various compositions offered on the market, we focused on fluoroplastic filter elements, which are not widely used in biotechnology today. But low cost of elements and good operational quality makes them competitive filters, the use of which in technological schemes of filtration seems to be optimal in terms of price - quality. The operational quality of these filter elements provides a long service life while ensuring high productivity.

The aim of the study was to investigate of the use of fluoroplastic filter elements in the production of a promising antibiotic substance pyocyanin.

Objects of investigation. Experimental technique

Porous polymeric materials were prepared at the Department of Materials Technology of the Institute of Technological Service the Kharkiv Petro Vasylenko National Technical University of Agriculture by preliminary preparation of a mixture of powders of the blowing agent and polymer, their mixing, pressing, heat treatment, leaching of the blowing agent and drying.

Powdered PTFE with a particle size < 100 μm and a density of $2.19 \cdot 10^3$ kg/m^3 was used as the basis of the porous polymer material. Sodium chlorine NaCl was chosen as a water-soluble blowing agent. The density of NaCl is close to that of PTFE and amounts to $2.16 \cdot 10^3$ kg/m^3, which makes it possible to obtain a high-quality mixing of the components [4].

Mixtures of PTFE and NaCl were prepared in a laboratory blender at a chopping knife speed of 2000 rpm for 60 s. The parameters of the processes of dispersion of the pore former, mixing of the components, the conditions for tableting, sintering and removal of the blowing agent are given in [5].

Substantiation of the possibility of using porous filter materials based on fluoroplastic was based on determining and comparing the number of cells of our selected model microorganism (*P.*

aeruginosa) in the culture fluid before filtration (control) and after filtration through the fluoroplastic material mounted in the syringe. The number of cells was determined by the traditional method of serial dilutions in microbiological practice and seeding on solid nutrient media [6]. Microbiological studies were carried out at the Department of Biotechnology of the National University of Pharmacy.

In parallel with all experiments, the purity of the culture was monitored, i.e. the presence of *P. aeruginosa* in the filtrate and the absence of foreign microflora (bacterioscopic control by viewing Gram-stained smears and bacteriological control by seeding on MPA and Saburo [6].

Results and discussion

The use of porous filter elements in industry is preceded by a large number of studies, first of all, determination the permeability or capacity of porous materials, due to the structure of the porous body.

Permeability is the basis of a physical quantity defined as the rate at which a liquid (or gas) passes through a unit area or unit thickness of a sample of material at a given pressure and is based on the application of Poiseuille's law to the flow of liquid through capillaries. It should be noted that according to this law, permeability is affected by many factors, primarily the composition of the liquid being filtered (its viscosity, density), inlet and outlet pressure, length and structure of capillaries or pores, and so on. Already on the basis of permeability is determined by the productivity of the material, the life cycle of the material, regenerating properties, etc. Also, many coefficients and indicators are determined experimentally for each case.

In this work we will dwell on the experimental substantiation of the possibility of using porous filter elements based on fluoroplastic by the microbiological methods.

The fluoroplastic filter elements are characterized by a long service life, i.e. their permeability is maintained over a long period of use, which is determined experimentally for each case, so we determined the number of cells *P. aeruginosa* after five tests. All experiments were performed in aseptic conditions of laminar box with sterile utensils and pre-treated syringes with built-in filter elements (fig.1).

Fig. 1. Separation of the culture fluid of *P. aeruginosa* using filtration through a porous filter element based on fluoroplastic in the laminar box

Determination of the number of cells of *P. aeruginosa* culture fluid before and after filtration through a porous filter element based on fluoroplastic is presented in Table 1.

Table 1

Determination of the number of cells of *P. aeruginosa* culture fluid before and after filtration*

The number of filtering processes performed through the same element**	Number of cells, CFU***/ml	
	After filtration	Control (before filtration) ****
1	117±3	$0,5\times10^9$
2	101±1	
3	108±5	
4	102±3	
5	96±7	
6	100±9	
7	108±10	
8	95±8	
9	100±10	
10	101±9	

Notes:

* – filtration fineness 1 μm, thickness h=6 mm;

** – area of the filter element (with contempt for the "dead" space formed by the superglue on the border of the syringe - the filter element); d = 19 mm;

*** - column-forming units; CFU was determined as the arithmetic mean value for 3 cups;

**** – the choice of initial concentration is determined by the turbidity standard of 5 units (respectively 0.5 billion cells in 1 ml)

(M±m) – confidence interval.

The number of *P. aeruginosa* colonies grown from a certain dilution of *P. aeruginosa* culture fluids before filtration and the number of colonies grown from a certain dilution after filtration are shown (Fig. 2). Visually, without the use of a microscope, we can see a significant reduction in the number of microorganisms after filtration.

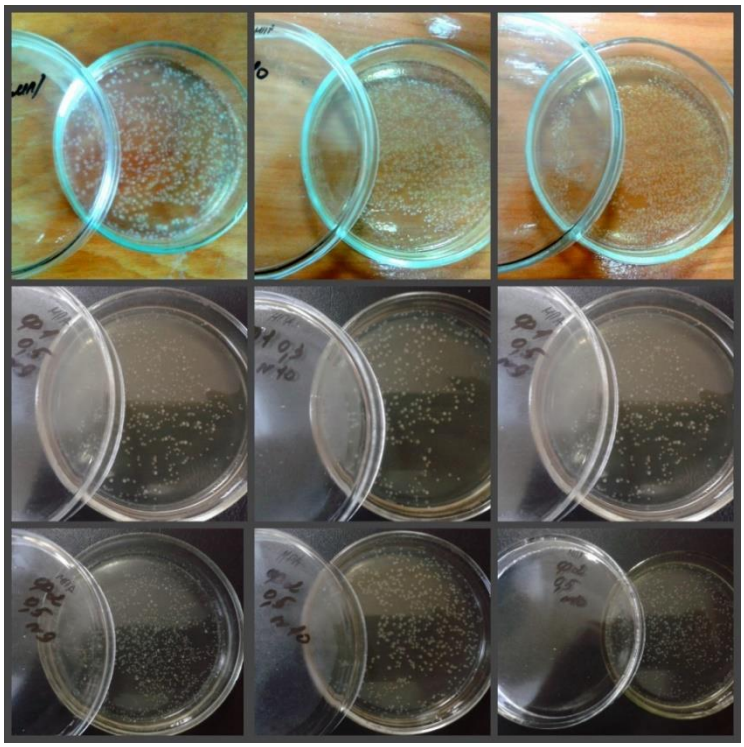

Fig. 2. The number of colonies of *P. aeruginosa* on the Petri dishes before filtration and after filtration

The results of Table 1 showed that in comparison with the control, the number of cells after filtration decreased significantly (about 10^6 times); it is shown that as the number of filtration

processes increases, the number of cells after filtration decreases, but this is not a statistically significant fact (this may be due to the formation of an additional filter layer of cells that spatially helped retain other cells).

It was also interesting for us to investigate the filtration of the formed filtrate through a new filter, i.e. in this case the initial concentration was the number of cells of *P. aeruginosa* in the previously obtained filtrate. The results are given in Table 2.

Table 2

Determination of the number of *P. aeruginosa* culture fluid cells before and after filtration of the previously obtained filtrate*

№ experiment**	Number of cells, CFU/ml	
	Before filtration***	After filtration
1	117±3	93±1
2	101±1	91±4
3	108±5	112±5
4	102±3	92±4
5	96±7	90±2
6	100±9	93±2
7	108±10	99±1
8	95±8	91±6
9	100±10	90±4
10	101±9	88±2

Notes:

* – filtration fineness 1 μm, thickness h=6 mm;

** – a new "fresh" filter element was used in each experiment;

*** - the number of cells in the filter, which was obtained in the previous series of experiments;

(M±m) – confidence interval.

The results of Table 2 show that at low concentrations of cells in the fluid (order 10^2 CFU), the filtered separation of cells from the culture fluid does not occur. This can be due to two facts:

- first, at such a low concentration does not form an additional filter layer, which would be an additional "spatial" barrier to the passage of cells;

- second, the sizes of *P. aeruginosa* cells vary from (1-5) × (0.5-1.0) microns and through the filter at the first filtration in a filtrate they got, therefore and at filtering through the new filter, without meeting any obstacles in the form of an additional layer, they again "passed" through the pores of the filter.

The next stage of our research was determination of the influence of the thickness of the filter element on the filtration efficiency.

According to the literature, it is known that the permeability of the filter element is affected by its thickness: theoretically, a thicker filter will have a larger pore surface, and when proving a certain diameter, which guarantees and is responsible for the manufacturer, it causes greater pore length, hence better "retention" properties of such filters.

In previous experiments, a porous filter element based on fluoroplastic with a standard TU thickness h = 6 mm was used.

To compare the efficiency of the filtration process with filter elements of different thickness, we were provided with 4 mm thick disks, with which we conducted a series of

experiments under the same conditions as above, but to save time and raw materials (nutrient media) we determined the number of cells after the first, fifth and the tenth filtration process, the results of which are given in Table 3.

Table 3

The number of *P. aeruginosa* cells in the filtrate after the n-th number of filtrations using filters of different thickness

The number of filtering processes carried out through the same filter	Number of cells, CFU**/ml	
	*Pseudomonas aeruginosa****	
	The thickness of the filter* h=6 mm	The thickness of the filter h=4 mm
1	117±3	$(98±7)×10^3$
5	96±7	$(101±13)×10^3$
10	101±9	$(94±8)×10^3$

Notes:
* - results of the previous series of experiments;
** - control is equal to 10^9 CFU/ml;
*** - fineness of filtration - 1.0 μm;
(M ± m) - confidence interval.

The results of Table 3 showed that the thickness of the filter element significantly affects the filtration efficiency. Regarding the filtration of *P. aeruginosa*, there is a significant increase in the concentration of cells in the filtrate (at least a thousand times), which indicates a decrease in filtration efficiency with decreasing filter thickness. Also, in the case of using a filter with a smaller thickness with increasing number of filtration processes the permeability of the filter element decreases in contrast to the use of filters with a thicker thickness.

Conclusions

1. Determination of filtration efficiency through fluoroplastic filters performed by separating *P. aeruginosa* culture fluid (filter with a fineness of 1 and 5 μm) from biomass and counting cell concentrations before and after filtration showed a significantly lower number of cells in the filtrate.

2. Carrying out series of filtrations (up to 10 times) everywhere the same filter showed preservation of permeability of a filter element and even some increase in efficiency of a filtration which we connect with formation of an additional filtering layer.

3. Filtration of *P. aeruginosa* culture fluid with a very low concentration of cells (about 100 CFU/ml) showed the presence of cells in the filtrate of the same order as before filtration. This is due to the size of the selected objects, ie there are shapes with much smaller sizes than the pore size. In addition, we should not forget about the lack of an additional filter layer of biomass of microorganisms formed during filtration.

4. The study of the influence of the thickness of the filter element on the filtration process (we used two dimensions - h = 4 mm and h = 6 mm) showed a significant increase in the efficiency of filtration with increasing thickness. This is due to the greater length of the pores, i.e. their larger surface area, hence the better "retention" properties of such filters.

Thus, the prospects for further research on the use of porous filters based on fluoroplastic in the production of antibiotic substances using the example of pyocyanin are shown.

Funding information

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflict of interest

The authors declare that they has no conflict of interest.

References

1. Centers for Disease Control and Prevention [Електронний ресурс] : [Інтернет-портал]. – Режим доступа: www.cdc.gov/drugresistance (дата звернення 05.09.2020). – Назва з екрана.

2. Pyocyanin: Production, applications, challenges and new insights /Sheeba Jayaseelan, Damotharan Ramaswamy, Selvakumar Dharmaraj // World Journal of Microbiology and Biotechnology. - 2013. - № 30(4) [Электронный ресурс]. URL: ttps://www.researchgate.net/publication/258431191 (дата звернення 05.09.2020).

3. Загальна (промислова) біотехнологія: навчальний посібник/ М.Д. Мельничук, О.Л.Кляченко, В.В.Бородай, Ю.В.Коломієць. – Київ: ФОП Корзун Д.Ю., 2014. - 252 с.

4. Kalyuzhny A.B., Karpova T.L., Kalyuzhny B.G., Platkov V.Ya. Structure and functional properties of high-porosity material based on Fluoroplast- 4 // Functional Materials. - 1999. - Vol. 6, №2. - P. 25-30.

5. Hogg R. Mixing and Segregation in Powders: Evaluation, Mechanisms and Processes. Kona Powder Part J 2009 27:3- 17.

6. Бактеріологія і вірусологія [Текст] : зб. нормат. док. / упоряд. та голов. ред. Заболотько Валентина Миколаївна; Головне упр. охорони здоров'я та мед. забезп., Київ. міськ. наук. інформ.-аналіт. центр мед. статистики . - К. : МНІАЦ мед. статистики: Медінформ, 2008 . - (Серія "Нормативні директивні правові документи"). - Ч. 1. - 2008. - 396 с.

References

1. Centers for Disease Control and Prevention [Electronic resource]: [Internet portal]. - Access mode: www.cdc.gov/drugresistance (date of operation 09/05/2020). - Name from the screen.

2. Pyocyanin: Production, applications, challenges and new insights / Sheeba Jayaseelan, Damotharan Ramaswamy, Selvakumar Dharmaraj // World Journal of Microbiology and Biotechnology. - 2013. - No. 30 (4) [Electronic resource]. URL: ttps: //www.researchgate.net/publication/258431191 (date of the beating 09/05/2020).

3. Zagalna (promislova) biotechnology: a master of knowledge / M.D. Melnichuk, O. L. Klyachenko, V. V. Boroday, Yu. V. Kolomiyets. - Kiev: FOP Korzun D.Yu., 2014 .-- 252 p.

4. Kalyuzhny A.V., Karpova T.L., Kalyuzhny B.G., Platkov V.Ya. Structure and functional properties of high-porosity material based on Fluoroplast-4 // Functional Materials. - 1999. - Vol. 6, no. 2. - R. 25-30.

5. Hogg R. Mixing and Segregation in Powders: Evaluation, Mechanisms and Processes. Kona Powder Part J 2009 27: 3-17.

6. Bacteriology and Virology [Text]: zb. norm. doc. / order. that heads. ed. Zabolotko Valentina Mikolaivna; Golovne exercise. protect the health of that honey. zabezp., Kiev. misk. sciences. inform.-analit. center honey. statistics. - K.: MNIATS honey. statistics: Medinform, 2008. - (Series "Normative directive legal documents"). - Part 1. - 2008 .-- 396 p.