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Two-stage bacteriophage production process supported by the electromagnetic field

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The practical applications of bacteriophages are associated with the problems related to the intensification, optimization of process production of this biomaterial and the search for new methods of production. The production of bacteriophages requires a fine balance between the dynamic growth of the bacteriophage and the host. The electromagnetic field (EMF) is a promising biotechnological method for the process production of bacteriophages. This study evaluates the use of various types of EMF to enhance the process. It was found that the process production of bacteriophages is divided into two stages. In the first stage, the influence of various types of EMF on the proliferation process of bacteria (host) was analyzed. Secondly, the process production of bacteriophage was implemented for the optimal infection conditions under the action of the various types of EMF. Moreover, the study demonstrated that the most effective bacteriophage production was the process with the application of the rotating magnetic field (RMF), pulsed magnetic field (PMF) and the static magnetic field (SMF) with negative polarity.

Keywords: bioreactor; biotechnology; bacteriophage production; electromagnetic field

1. INTRODUCTION

Bacteriophages (also known as phages, viruses that infect bacteria) are the most abundant biological entities in our biosphere (Kropinski, 2018). Phages are made up of protein or proteolipidic capsides containing fragments of nucleic acids, and these creatures have evolved multiple strategies to interfere with bacterial growth. These biological entities can be harmless for all organisms, including humans, except for their target bacterial host (Harada et al., 2018). Frederick Twort first described the characteristic zone of lysis associated with phage infection in 1915, but it was Felix d'Herelle who identified the source of this phenomenon (Lin et al., 2017). Felix d'Herelle used bacteriophages in clinical application in 1919 to treat

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four pediatric cases of bacterial dysentery (Chanishvili, 2012). Up to now, there has been much research directly investigating the production of bacteriophage. Bacteriophage research development is graphically shown in Fig. 1.

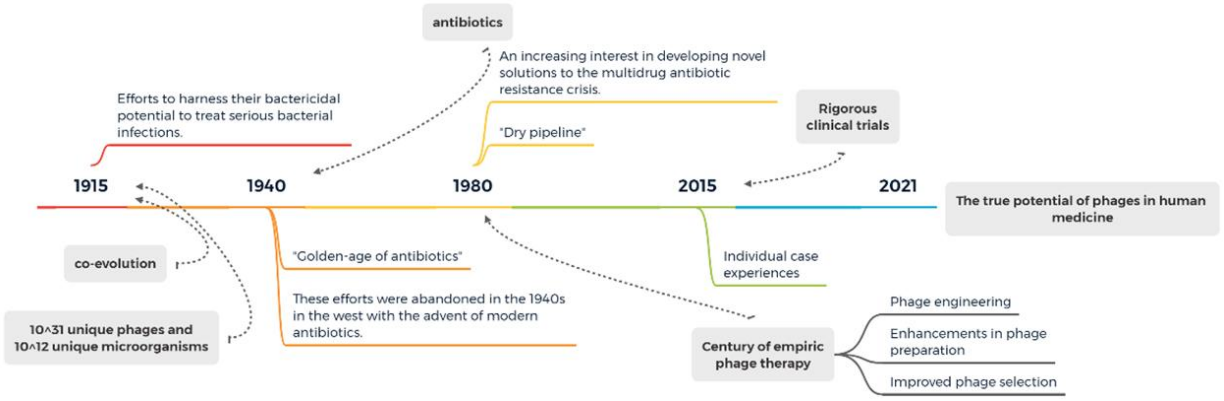

Fig. 1. Schematic representation of bacteriophage research evolution

The general method for phage production is based on the application of bioreactors (Agboluaje and Sauvageau, 2018). Historically, research connected with the production of bacteriophage has focused on applying the vessel with an impeller (Sargeant et al., 1968). These investigations showed that the use of the novel type of impeller improved the oxygen solution rate in 20 dm^3 vessels. Werquin et al. (1984) found that the performance of a fermenter for the aerobic process was governed by the efficiency of the apparatus in transferring oxygen from the air to the liquid medium and forming this to the microorganisms (in this study a fermenter of 4.5 dm^3 capacity was used). Grieco et al. (2009) examined computer-controlled bioreactors to produce large quantities of filamentous phages with the use of the *Escherichia coli* as a bacterial host (3 dm^3 Applikon bioreactors). One study by Grieco et al. (2012) examined the optimal fermentation conditions by means of the three key process parameters (pH, temperature, and dissolved oxygen). Smrekar et al. (2011) used biostat to cultivate bacteriophages. In the case of these experimental results, the monolithic column was applied for the purification of the medium. A stirred-tank bioreactor was also used to produce phages (Ferrara et al., 2015). The titers obtained in this type of bioreactor were 4 to 5 times higher than in a standard shake flask procedure. The process production of bacteriophage for constant and variable conditions using cellstat was discussed by Nabergoj et al. (2018).

Previous research has established that the conditions for optimal production of bacteriophage are not the same as the conditions for optimal host growth. The maximum bacteriophage amount is connected with the theoretical maximum of the host concentration (Agboluaje and Sauvageau, 2018). The production of bacteriophages requires a fine balance between the dynamic growth of the bacteriophage and the host (Hadas et al., 1997). It should be noticed that many factors affect population dynamics, e.g., host-growth rate, metabolic activity, temperature, pH, medium composition, aeration rate, agitation, and the presence of ions or cofactors (Jończyk et al., 2011). It should also be noted that the bioreactor systems play an important role in establishing the infection and operating conditions that will influence optimal bacteriophage titers. Previous studies have reported that the production of bacteriophage T7 was carried out in 3 dm^3 or 20 dm^3 culture vessels with an impeller and the flow rate of a sterile air (Sargeant et al., 1968). The interaction between bacteriophage λ and bacteria (*Escherichia coli*) was studied in different bioreactor systems (0.25 dm^3 shake flasks and 1 dm^3 BioFlo bioreactors) (Chen and Lim, 1996).

Several researchers have reported that the general types of bioreactors or bioreactor arrangements used in the production of bacteriophages were batch, continuous, two-stage continuous, and two-stage cycling (Agboluaje and Sauvageau, 2018; Sauvageau and Cooper, 2010). Table 1 shows the characteristics of the used bioreactor systems in the production of bacteriophages.

Table 1. The characterization of bacteriophage production with the application of various bioreactor systems

Bioreactor systems used in bacteriophage production	Characterization	Refs.
Batch system	• Used in most production schemes of bacteriophage. • The infection is initiated at a given multiplicity of infections (MOI, ratio of virus particles to bacteria host-cells). • The host-cells and phage populations grow until the viruses cause population-wide lysis. • Dependent on the initial and operational conditions. • Carried out using bioreactors with various volumes equipped with the stirrer.	(Agboluaje and Sauvageau, 2018) (Sauvageau and Cooper, 2010)
Single-stage continuous bioreactor system	• Based on the application of the chemostat and the turbidostat. • The cultivation process is carried out at high volumetric throughput. • Not recommended for the bacteriophage production (e.g. the rise of mutations and host-phage coevolution; low quality of product). • Difficult to operate in steady-state conditions. • The residence time distribution in bioreactors and the threshold population plays an important role in the continuous production of bacteriophages.	(Williams, 2012) (Mizoguchi et al., 2003) (Wichman et al., 2005)
Multi-stage continuous bioreactor system	• More effective than batch and single-stage continuous systems. • Relies on the host grown in the absence of the bacteriophage in a first bioreactor and sent to a second bioreactor for infection. • Improvement of the threshold population densities (greater titers than during the single-stage operation). • Can be operated under different sets of conditions, e.g. the optimal growth in the first stage; the optimal infection in the second stage.	(Oh et al., 2005) (Chen and Cen, 2005)
Two-stage semi-continuous bioreactor operation	• The host is grown in the first stage and infection proceeds in the second stage (in this case the semi-continuous bioreactor systems are used). • The small fraction of bacteriophage produced in the previous cycle is used to infect the host fed from the first bioreactor (it allows to obtain the high titers of process, high throughput, and smaller downtime to production time). • Residence time distribution is proper to kill all host cells (the probability of coevolution is smaller).	(Sauvageau and Cooper, 2010) (Storms et al., 2014)

Oh et al. (2005) conducted a series of experiments examining various bioreactor arrangements (batch operation, single-stage continuous operation, two-stage continuous culture). The results indicated that the two-stage continuous culture allowed to obtain the maximum process production. The two-stage self-cycling process for producing bacteriophages was also tested by Sauvageau and Cooper (2010). This study focused on developing a cycling (semi-continuous) two-stage process for bacteriophages (*Escherichia coli*/T4 system was used as a model system). The first stage (the host cells are grown) was operated under the principles of self-cycling fermentation. The second stage (the host is infected by the phage) was operated in an automated cycling mode. Synchronization of the host in the first stage before infection led to improvements in the specific productivity of phages in the second stage while maintaining the volumetric

productivity. Podgornik et al. (2014) explained the process of bacteriophage multiplication and implemented it in their continuous production in bioreactors. Moreover, this work showed the mathematical model of production of bacteriophages in the continuous regime. The computational models of bacteriophage production with a continuous chemostat are described by Sulakvelidze et al. (2018). Krysiak-Baltyn et al. (2018) developed a computational model to simulate the dynamics of phage population growth and production in a two-stage, self–cycling process. The kinetics of the two-stage continuous production of bacteriophage was modelled by Park and Park (2000).

The increasing number of potential bacteriophage applications or virion parts is most likely to increase the demand for new technologies supporting the industrial production of bacteriophages in the nearest future. For the intensification of biotechnological processes, various methods to stimulate living organisms are frequently used (Domingues et al., 2000). The application of force fields (electric or magnetic field, or ultrasounds) to intensify biomass production seems to be particularly interesting. Rosensweig was the creator of the concept of the magnetic bioreactor and the precursor of microbial growth stimulation with the use of electromagnetic support for the bioprocess (Rosensweig, 1979). The magnetic field is used to intensify the production of biomass, as well as for intensifying biochemical processes and enzymatic reactions (Al-Qodah et al., 2017; Wang et al., 2017; Zhang et al., 2017).

The application of electromagnetic field (EMF) to increase the process production of induced lambdoid bacteriophages was discussed by Struk et al. (2017). Gained results showed that exposing cells to mitomycin C together with EMF led to the enhancement of the production of bacteriophages. It should be noted that the adsorption process of T4 bacteriophage on the bacteria host's surface could be improved by rotating magnetic field (RMF) exposition (Grygorcewicz et al., 2022).

Bacteriophages have now been identified as important tools in nanomedicine, e.g., as phage display for treatment or drug discovery, gene or drug delivery, or indirect cancer treatment (Rao and Zhu, 2022; Sivaperumal and Kamala, 2022). Moreover, bacteriophages are used obviously in phage therapy, detection and diagnostic, bacterial control and typing, or recombination protein production (Byeon et al., 2015; Hiremath et al., 2015; Wang et al., 2016; Winton, 2015). These developments have led to re-evaluating the potential biotechnological applications of these metabolically inert entities and to attempts to improve production methods. Therefore, the main aim of this investigation was to show the novel approach to the production process of bacteriophages. The two-stage batch method relies on the application of the various types of electromagnetic fields in the growth of the host (*Klebsiella pneumoniae* bacteria) and the process of production of vB_KpnM_Kpn0346 phage, a lytic *K. pneumoniae* infecting bacteriophage.

In the first stage of bacteriophage production, the impact of various types of electromagnetic fields (EMF) on the proliferation process of bacteria was tested. Moreover, this study aimed to find optimal thermal and EMF conditions for the process production of *K. pneumonia* biomass in the magnetically assisted bioreactor. Secondly, the production of bacteriophages was carried out for the optimal infection conditions that would lead to the highest titer with the least amount of host cell debris. It should be noted that the second stage of the proposed method of production of bacteriophage was also realized using the various types of EMF. It allowed us to test which conditions had a positive effect on the production of bacteriophages. In the proposed method, a large infection load is used with a high initial MOI. The rapid infection of a large portion of the host population and obtaining the swift population-wide lysis is the main goal of this approach. It should be emphasized that the general strategy adopted in this method is to decouple the growth of the host (*K. pneumonia* biomass) from the infection process. Briefly, the host is grown in the absence of the bacteriophage in a first magnetically assisted bioreactor (stage 1) and then sent to a second magnetically assisted bioreactor for infection (stage 2). Initially, the optimal temperature and parameters connected with the application of EMF are selected for the process production of biomass. For these conditions the infection of the host by bacteriophage is carried out. The proposed method of production of bacteriophages is novel, and this approach has not been reported before.

2. MATERIALS AND METHODS

2.1. Experimental set-up

The two-stage production of bacteriophages supported by the electromagnetic field (EMF) is carried out by using the magnetically assisted bioreactor. The main part of this experimental set-up (see Fig. 3) is the generator of EMF, which produces a direct current magnetic field (DCMF) and alternating current magnetic field (ACMF). The magnet assembly consists of three identical coil pairs, spatially shifted from each other symmetrically. Each coil can be energized separately or coupled to other coils in various configurations. Therefore, the generators can be used to produce different types of EMFs, including static magnetic field (SMF), oscillating magnetic field (OMF), and rotating magnetic field (RMF). It should be noticed that the pulsing magnetic field (PMF) was generated by the application of intermittent power supply to the coils in the fixed configuration of winding connection for OMF.

The connection diagram of windings in the used generator of EMF is presented in Fig. 2.

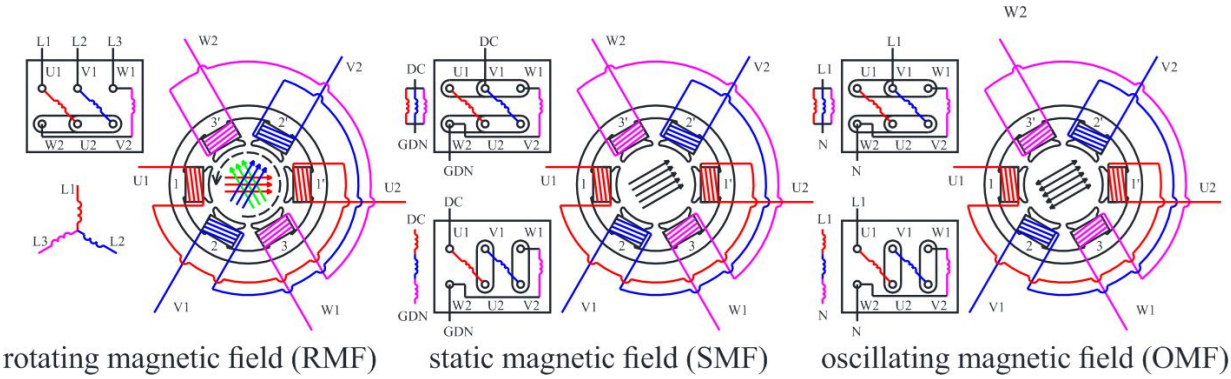

Fig. 2. The connection diagram of windings in the used generator of EMF

A brief characteristic of each EMF type analyzed in the current study is presented in Table 2.

Table 2. The characterization of electromagnetic fields used in current work

EMF type	Characteristics
Static (SMF)	• Generated by using DC-powered circuits • Highly uniform force field in the region between the coils • Characterized by polarity, commonly called north and south poles, which attract opposite charges
Oscillating (OMF)	• Generated by AC-powered circuits • Varies in time with some frequency • Possesses an external MF vector which changes as a sinewave with time at each point of the space
Pulsating (PMF)	• Specific case of OMF • Generated by the manipulation of power of electric circuits during the exposure • Could create intermittent occurrences of EMF with the empty gap between each pulse, or they can change regularly to create some homopolar EMF
Rotating (RMF)	• Generated by a set of symmetrically placed coils around the central axis • Constant intensity over time while it changes its direction continuously at any point of the domain • Created in the stator and rotor windings of an induction motor by the superposition of three 120°out of phase OMFs

The sketch of the experimental setup is given in Fig. 3.

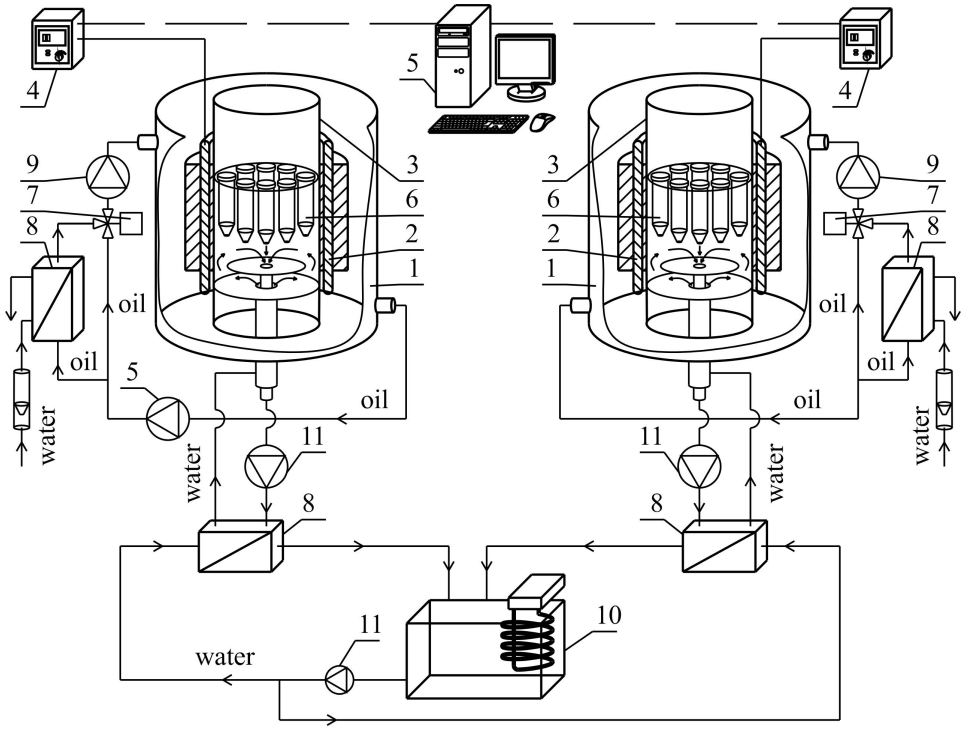

Fig. 3. Sketch of the experimental setup: 1 – housing; 2 – generator of EMF; 3 – process chamber; 4 – transistorized inverter or power supply; 5 – computer; 6 – probes; 7 – three-way valve equipped with actuator; 8 – heat exchanger; 9 – circulating pump for oil; 10 – thermostat; 11 – circulating pump for water

The experimental set-up consisted of two magnetically assisted bioreactors (MABs) equipped with the generators of EMF (in the case of these investigations, SMF, OMF, PMF, and RMF were tested), which could work separately. In the case of this experimental work, one MAB was used to carry out the two-stage production of bacteriophage under the action of various types of MF. The second MAB served as the control setup (without the action of EMF).

Each reactor consisted of a housing (1) and the generator of EMF (2). The cylindrical process chamber (3) was axially aligned with the EMF generator (2). This generator was connected to the transistorized inverter or power supply (4) and computer (5). The probes (6) were placed in a water bath at the established temperature. The RMF generator during work produces heat due to the electric resistance of coils powered by DC or AC. Therefore, the generator is submerged in silicone oil (an electric isolator) which allows transporting the heat outside the tank by the oil circulation pump (9). Excessive heat can be removed from the system through the plate heat exchanger (8). The amount of heat within the water stream transported back to the tank is controlled by the control valve (7) equipped with the temperature sensor. The circulation pump (11) was forced to flow the water between the chamber of the reactor and the heat exchanger. To establish the stable temperature of the liquid inside the rector, the working liquid (water circulating between the reactor and the heat exchanger) was heated in the counter-current heat exchanger (8) by the water from the thermostat (12). This system ensured the constant temperature also for the reactor with control samples.

2.2. Characterization of the applied EMF

The values of magnetic induction inside the cylindrical chamber were detected using the Gaussmeter (FW Bell 5180 magnetometer G-meter; Magnetic Science Inc., United States) with the measurement accuracy equal to ±2.5% of the reading. The magnetic induction measurements for SMF, OMF, PMF, and RMF

were carried out in the active area of the generator of EMF. The detailed methodology of measurements was presented in the previous paper (Jabłońska, et al., 2022b). Table 3 shows the experimental data on the magnetic induction of the tested types of electromagnetic fields (EMF).

Table 3. Characteristics of the applied various types of EMF

Type of EMF	Range of changes of operating parameter	Values of magnetic induction	Characteristic
SMF	For parallel connection of coils: V = 1–12 V For series connection of coils: V = 5–45 V	For parallel connection of coils: B = −18.4–24.9 mT For series connection of coils: B = −27.6–36.1 mT	1. In the case of SMF, the voltage power supply was used as the operating parameter (V). 2. The SMF is described by the minimum and maximum values of magnetic induction. 3. This type of magnetic field is characterized by polarity (magnetic induction takes positive and negative values).
RMF	f = 1–50 Hz	B_{max} = 12.3–42.6 mT B_{avg} = 7.3–19.9 mT	1. In the case of the RMF and OMF, the frequency of the current supplied to the generator windings (f) was treated as the operating parameter. 2. In the case of this experimental work, the RMF and OMF was characterized by means the maximum and the averaged values of the magnetic induction. 3. In the case of OMF and SMF, the windings of generator can be connected in series or in parallel.
OMF	For parallel and series connection of coils f = 45–65 Hz	For parallel connection of coils: B_{max} = 5.1–7.7 mT B_{avg} = 1.1–1.5 mT For series connection of coils: B_{max} = 15.7–22.6 mT B_{avg} = 3.7–5.3 mT	
PMF	For parallel and series connection of coils f = 45–65 Hz with On-Off mode		1. The PMF is the modification of the OMF. This type of magnetic field was created by the application of an intermittent power supply to the coils (On-Off mode). 2. In the case of these investigations, the On-Off mode was fixed as follows: a) On (1 s)-Off (1 s); b) On (10 s)-Off (10 s); c) On (60 s)-Off (60 s); d) On (600 s)-Off (600 s).

2.3. Two-stage production of bacteriophages

The general strategy adopted in these cases is to decouple the growth of the host from the infection process. The host is thus grown in the absence of the bacteriophages in a first bioreactor (stage 1) and sent to a second bioreactor for infection (stage 2). The first and second stages are carried out under the action of the various types of EMF.

The first stage of this process is the selection of the optimal conditions for biomass production. Therefore, the optimization of the *K. pneumoniae* growth conditions was planned with the design of the experiment (DoE) technique utilizing the central composite design (in the case of this work, Statistica 13.3 software was applied to obtain the experiment plan). In this study, we decided to test bacteria growth under various types of EMF exposure at multiple temperatures. For the accepted conditions of the experiment, the bacterial growth was monitored using optical density (OD) measurements. The Gompertz function Pole described the obtained curves (Winsor, 1932).

Moreover, the maximum growth rates were calculated for the obtained results (Jabłońska et al., 2022a). These values were a function of the temperature and the magnetic induction (parameter describes the applied EMF). The values of adjusted Gompertz function parameters and calculated μ_{max} values are presented in Table A1 in the Appendix. Therefore, for the following function

$$\mu_{max} = f(T, B) \tag{1}$$

was used to estimate accurate optimal temperature and parameter that described the applied EMF. To find the optimal conditions of the process production biomass (which means that the maximum growth rate has the maximum value), we used the software Design Expert v13 (Stat-Ease, US). Design Expert provides built-in tools for the best selection of the design of experiment, statistical analysis and visualization of the results. Moreover, it provides the response surface methodology to optimize selected parameters, through the mathematical description, factor analysis, and surface plot to the automatic multi-parameter function optimization.

2.4. K. pneumoniae cultivation parameters (the first stage of the process)

The present studies used a reference strain of *K. pneumoniae* ATCC BAA-1706. Overnight cultures cultivated at 37 °C were used as inoculum (1:100) and dispensed to Falcon tubes (10 mL of inoculum to each tube). Starting from $t = 0$, samples (100 µL each) were taken to measure optical density (OD, at $\lambda_{OD} = 600$ nm) on BioTek Synergy H1 (Winooski, VT, USA) spectrophotometer. The experiments were continued in aerobic conditions to achieve the stationary phase. At each time point, a sample for OD measurement and resazurin-based cell metabolic activity was taken from each bioreactor. Resazurin assay was prepared by loading 10 µL of resazurin (1 mg/mL in PBS) to each well. Afterwards, the samples were incubated at 34 °C for 20 min, and the fluorescence ($\lambda_{ex} = 520$ nm and $\lambda_{em} = 590$ nm) was read on the spectrophotometer BioTek Synergy H1 (BioTek, Winooski, VT, USA). The cultivation was led in various temperatures and electromagnetic field frequencies given by the experiment's design and control temperature conditions (without the electromagnetic field marked as $f = 0$ Hz). Moreover, to confirm gained tendencies, all experiments were triplicated. Within each experiment, all harvested samples were studied in eight repetitions.

2.5. Bacteriophage cultivation parameters (the second stage of the process)

Batch infections were performed with magnetic field-supported fermenters. The phage production conditions were selected based on previous analysis optimizing the *K. pneumoniae* biomass production. The phage infection was started with approx. 1×10^8 CFU of *K. pneumoniae* and phages were added to the final initial MOI = 0.01. Phage productivity was monitored as an endpoint with the use of the double agar layer assay and calculated as:

$$P = \frac{C_{EMF}}{C_{Cont}} \tag{2}$$

3. RESULTS AND DISCUSSION

As mentioned above, the first stage of the process production of bacteriophage was the production of the host's biomass (in the case of these investigations, *K. pneumonia* bacteria was the host). This process was carried out for the various temperature values and the selected operating parameters of the applied EMF (see Table 2). Moreover, the optimization of the *K. pneumonia* growth conditions was carried out using the design of the experiment (DoE). The application of the software Design Expert v13 (Stat-Ease, US) allowed to obtain the experiment designs. Two parameters were selected for optimization, i.e., temperature (T) for all types of the tested EMF and frequency for RMF and OMF or the voltage for SMF. In the case of PMF, the time duration of electromagnetic pulses was considered.

Based on the software Design Expert v13 capabilities, the schemes of experiment designs for the tested conditions and types of EMF were established. The figure below (Fig. 4) illustrates the scheme of the experiment design for the host's biomass production under the action of the RMF.

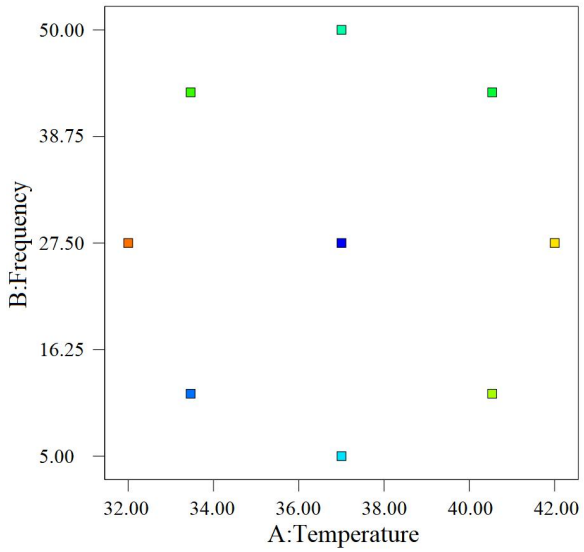

Fig. 4. The scheme of the experiment design for the host's biomass production under the action of RMF (this process was conducted for various temperature values and frequencies of power current of generator windings)

For the selected parameters, the bacterial growth was monitored using optical density (OD) measurements. Figure 5 shows the typical changes of the OD for bacterial growth under the different temperature conditions (without the application of EMF).

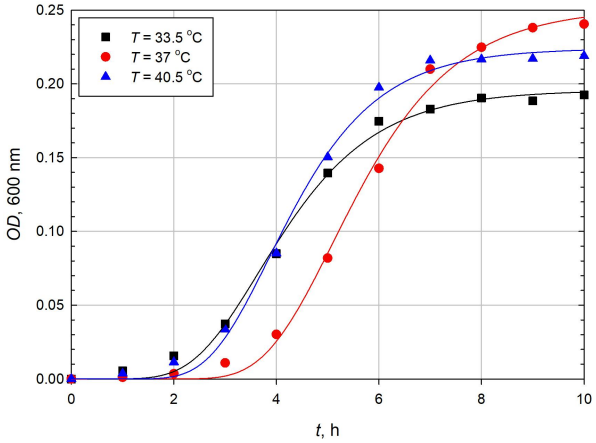

Fig. 5. The typical growth curve of *K. pneumonia* for the various temperature and without the application of EMF (the solid line shows the approximation using the Gompertz function)

As shown in Fig. 6, the application of RMF (for the steady temperature conditions of cultivation) strongly influences bacterial growth.

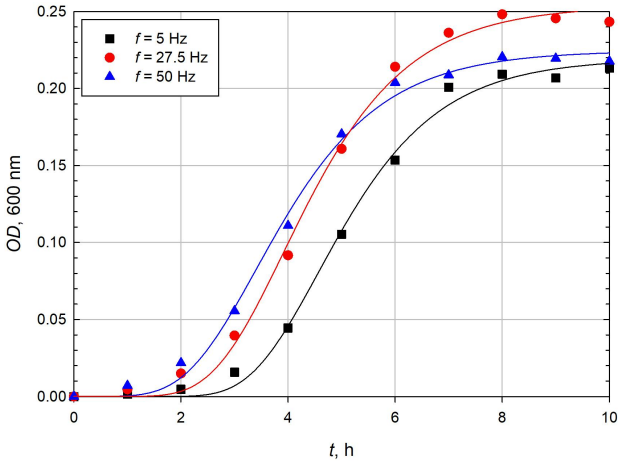

Fig. 6. The typical growth curve of *K. pneumonia* for the constant temperature (T = 37 °C) and with the application of RMF (the solid line shows the approximation using the Gompertz function)

The obtained scatters of the experimental points (OD values) were described using the Gompertz model. It was used to model the bacterial growth and to calculate the maximum specific growth rate (Winsor, 1932). A detailed description of the calculation method of this parameter of the growth curve was described previously (Jabłońska et al., 2022a).

The values of the maximum growth rate (μ_{max}) were correlated as a function of the temperature and the parameter describing the used type of EMF. These parameters might be then optimized using the Response Surface Methodology (RMS) which is a widely used analytical method for optimization (this option is a built-in tool in the applied software Design Expert v13). The main aim of this method is to determine the interaction between the independent variables, alone or in combination, in the form of a multi-parameter mathematical model. This model generates an accurate mathematical description of the overall process and may be used to analyze the effects of the independent variables. The RMS method describes the interactive effects of process variables on the process in the form of graphical representation. The application of RSM allowed to select the optimal experimental conditions requiring the lowest number of experiments to obtain appropriate results.

Figure 7 provides the results obtained from the analysis of the obtained values of the maximum specific growth rate as the function of the temperature and the frequency of RMF (Fig. 7a). Looking at Fig. 7b, it is apparent that the maximum value of μ_{max} was obtained for the temperature and frequency equal to 34.6 °C and 24.5 Hz, respectively.

Figure 8 presents the obtained results of the RSM analysis for the OMF. What stands out in this figure is that the maximum values of μ_{max} for the process production of the host's biomass under the OMF conditions were obtained for the temperature and frequency of the OMF equal to 34.2 °C and 64.7 Hz, respectively.

A similar procedure for determining the maximum specific growth rate was carried out for the other types of EMF. For each analysed EMF type a set of the optimized operating parameters: temperature and EMF-connected parameters (for the highest achieved values of maximal specific growth rate related to the control process, without EMF exposure) was found. The results of the optimization procedure for the tested types of EMF are given in Table 4.

The effect of exposure to the electromagnetic field during the production of bacteriophages depends on the type of field used. The most effective was the production stimulation with the RMF, thus allowing

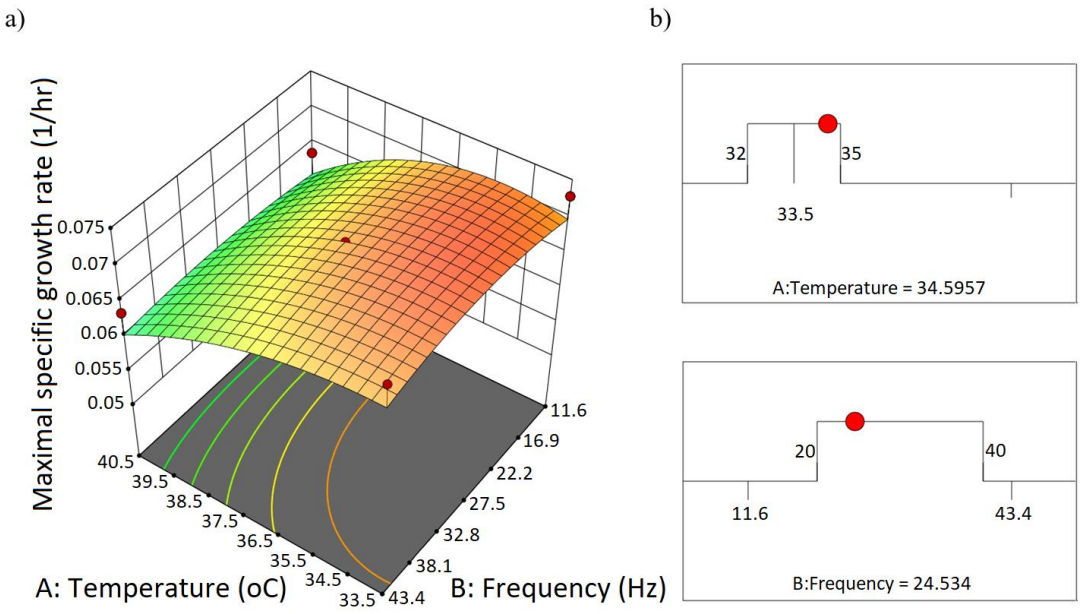

Fig. 7. The result of the optimization procedure with the application of the software Design Expert v13 for biomass process production under the action of RMF and various values of temperature: a) the RMS for the maximum growth rate as the function of temperature and frequency; b) the obtained values of the optimized operating parameters (temperature and frequency of RMF) for which the value of μ_{max} is maximum)

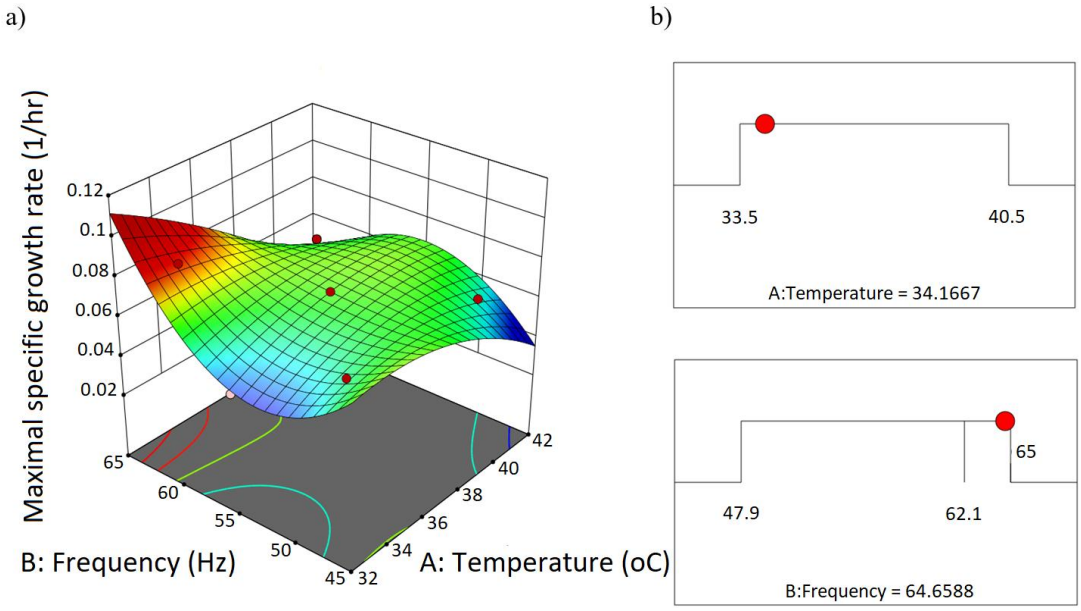

Fig. 8. The result of the optimization procedure with the application of the software Design Expert v13 for biomass process production under the action of OMF and various values of temperature: a) the RMS for the maximum growth rate as the function of temperature and frequency; b) the obtained values of the optimized operating parameters (temperature and frequency of OMF) for which the value of μ_{max} is maximum)

for 126.3% increase of productivity observed in the control reactor. Another highly efficient process was the exposure to the PMF, thus allowing to obtain approx. 120% increase of productivity. Using SMF with negative polarity (samples placed at the N-pole of magnetic field) allowed to obtain 112% bacteriophage production increase compared to the control reactor. For the other fields used, no increase or decrease in the number of daughter virions was observed during the production process (Figure 9).

Table 4. The optimal values of parameters for the first stage

Type of EMF	Value of operating parameter	Value of magnetic induction	Temperature	Ratio $\frac{\mu_{max}\|_{EMF}}{\mu_{max}\|_{control}}$
SMF	For series connection of coils V = 25 V	B = +20.9 mT	34.4 °C	1.15
		B = −15.3 mT	34.4 °C	1.11
RMF	f =24.5 Hz	B_{max} = 37.1 mT B_{avg} = 18.4 mT	34.6 °C	1.10
OMF	For series connection of coils f = 64.7 Hz	B_{max} = 15.7 mT B_{avg} = 3.7 mT	34.2 °C	1.07
PMF	For series connection of coils f = 64.7 Hz Pulse duration 1 s	B_{max} = 15.7 mT B_{avg} = 3.7 mT	34.2 °C	1.09

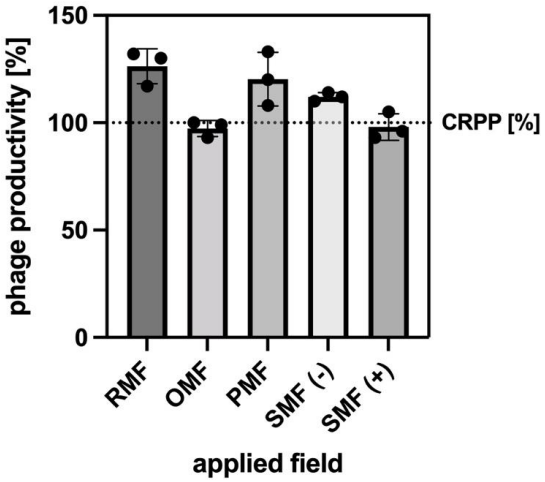

Fig. 9. Productivity of phage particles under various types of electromagnetic field exposure for *K. pneumoniae*. CRPP – control reactor phage

The future use of bacteriophages, both in medicine and in many branches of industry, forces the development of methods to increase the productivity of bacteriophages (Augustyniak et al., 2018; Grygorcewicz et al., 2020; 2021; Kaźmierczak et al., 2022). Previous work showed that it is possible to increase the productivity of lambdoid bacteriophages due to exposure to a rotating electromagnetic field after induction with mitomycin (Struk et al., 2017). Additionally, as shown by the optimization results, it is possible to accelerate the growth of the bacterial culture using electromagnetic stimulation. Similar results have been shown before. Bacterial cells that grow and metabolize faster are usually more susceptible to attack by bacteriophages. In addition, the intracellular development of phages is directly related to the physiological state of the bacterial cell (Hadas et al., 1997; Struk et al., 2017). Another factor that may influence the development of cells and thus bacteriophages is the phenomenon of micro-mixing. This phenomenon may lead to an increase in the amount of dissolved oxygen in the medium, thus increasing its availability to the cells (Rakoczy et al., 2017; 2018). Micro-stirring can also affect the mobility of ions. It was observed that the cells after RMF exposure had changed their membrane and cell wall potential, which, together with the change of the zeta-potential of bacteriophages, increased the effectiveness of their adsorption to the

cell. Changes in the cell charge to more negative may also promote adsorption of positively charged ions and nutrients which may influence the growth of bacteria (Beebout et al., 2021; Grygorcewicz et al., 2022; Konopacki and Rakoczy, 2019).

4. CONCLUSIONS

The main goal of the current study was to determine a novel production process of bacteriophage. This study set out to investigate the impact of the various types of EMF on the production of bacteriophages. The findings indicate that this process might be carried out using the two-stage approach. The experiments confirmed that the magnetically assisted bioreactor might be used to obtain the optimal condition of the proliferation process of bacteria (the first stage of the proposed process). The present study establishes that the production of bacteriophages for the optimal infection conditions leads to the highest titer with the least amount of host cell debris (second stage of the proposed process). This stage was also carried out using the various types of EMF. The RMF, PMF and SMF with negative polarity are types of EMF which increased the efficiency of bacteriophage production. A key strength of the present study was the proposed novel method of bacteriophage production. It was the first study that found that this process could be conducted in two stages. These findings highlight the potential usefulness of various types of EMF in the production of bacteriophages.

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SYMBOLS

B	magnetic induction, mT or $kg \cdot s^{-2} \cdot A^{-1}$
C	concentration of phages, PFU/ml
f	frequency of current flowing through the coil, Hz or s^{-1}
P	phage productivity, %
T	temperature, °C or K
t	time, s or h
V	electrical voltage, V or $kg \cdot m^2 \cdot s^{-3} \cdot A^{-1}$

Greek symbols

μ_{max}	maximum growth rate, h^{-1} or s^{-1}

Subscripts

avg	averaged value
Cont	control experiment
max	maximum value
EMF	experiment under electromagnetic field exposure

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APPENDIX

Table A1. Adjusted parameters of Gompertz function and calculated μ_{max} values for each conducted experiment

T [°C]	f [Hz]	a	b	c	R^2	μ_{max} [1/h]
32.0	0.0	0.2598	4.1776	0.6808	0.9972	0.065070
32.0	27.5	0.2748	3.8216	0.6636	0.9981	0.067085
33.5	0.0	0.2543	3.8010	0.7463	0.9979	0.069815
33.5	11.6	0.2822	2.8473	0.7007	0.9975	0.072742
33.5	43.4	0.2811	2.8244	0.7013	0.9975	0.072525
37.0	0.0	0.2278	2.9948	0.7689	0.9976	0.064434
37.0	5.0	0.2251	3.6350	0.7928	0.9979	0.065649
37.0	27.5	0.2533	2.9252	0.7609	0.9976	0.070900
37.0	50.0	0.2256	2.5463	0.7522	0.9967	0.062429
40.5	0.0	0.2019	2.6220	0.7115	0.9974	0.052850
40.5	11.6	0.2024	3.0085	0.8526	0.9976	0.063483