APPLIED PHYSICS

Physical Methods of Disinfection (A Review)

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Abstract—The basic physical methods of disinfection of air, water, and surfaces, such as filtration, ozonation, exposure to ultraviolet radiation, photocatalysis, cold plasma, electric discharges, and electroporation in an electric field are reviewed. The main attention is paid to the consideration of traditional and new methods of air disinfection. Recommendations on application of pulsed UV radiation are given. The possibilities of the electric field application for water and air disinfection are analyzed.

Keywords: disinfection, microorganisms, filtration, UV radiation, electric discharge, photocatalysis, plasma medicine, electroporation

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1. INTRODUCTION

For a long time, oxidizing technologies with the use of chlorine and other chemical disinfectants have been used to disinfect and sterilize water and surfaces. These chemical technologies were not used for air, since most chemical disinfectants are either poisonous or have extremely negative effects on human health when inhaled with air. Long-term domestic and foreign medical studies of the effect of chemical disinfectants on the health of the population show a stable correlation between diseases of the respiratory system, digestion, inflammation of the mucous membranes and the content of chemicals used in the atmosphere. In the 1970s, it was also discovered that by-products formed during the chlorination of water, mainly halogen-organic compounds, in drinking water pose a danger to human health, and when in wastewater, they cause serious damage to the ecology of water reservoirs. At the same time, chlorination and other oxidative disinfection technologies are ineffective against viruses.

Despite the fact that the history of water disinfection has been going on for two centuries, at present new methods are being developed, and especially actively physical methods, including well-known methods, e.g., the use of ultraviolet (UV) radiation are being improved [1].

For a long time, air disinfection has been carried out mainly in medical institutions, and only recently it has begun to be used in transport, in retail and office premises. There are objective reasons for this trend. Airborne infections are one of the most urgent problems of infectious safety. The emergence of new dangerous types of infections transmitted by airborne droplets, the presence of bioterrorism threats, the transportation of infected passengers in crowded cities, the possibility of transporting infections over long distances in a short time, e.g., by air, increase the risk of spreading infectious diseases. In turn, this increases the urgency of improving the existing methods of air disinfection and developing new ones. Disinfection of air is necessary in places of mass stay of people: hospitals, public transport, train stations, school institutions, theaters, indoor sports complexes, etc. Disinfection in medical institutions remains a problem. From 5% to 10% of patients admitted to modern hospitals in developed countries get a nosocomial infection [2, 3]. In this case, air and surfaces are one of the main factors in the transmission of nosocomial infections [4].

Disinfection of indoor air has its own features: the difficulty or impossibility of using chemical disinfectants in the presence of people, rapid mixing and overflow of air in different rooms, the presence of sources of infection in the rooms (sick people) and the possibility of air re-infection [4]. The use of chemical reagents for disinfection leads to an unjustified increase in the chemical load on the human population. Unlike industrial chemical pollution, disinfectants are introduced directly into the human environment and their use is strictly limited by the standards for the residual content of sterilizing agents.

Due to the emergence of new methods of disinfection that were not previously used in everyday practice, as well as active advertising of new technologies and their new possibilities, sometimes consumers face a difficult choice, especially for disinfection in critical places, e.g., in hospitals, operating rooms and postoperative wards, etc. This issue becomes especially complicated when choosing methods and equipment for air disinfection, when it is necessary to take into account the efficiency and reliability of disinfection, room size, treatment duration, ease of operation, equipment cost, size and weight, and other factors.

With allowance for the above, the purpose of this work is a comparative consideration of physical methods of disinfection (sterilization) of air, water and surfaces, both traditional and relatively new ones.

2. BASIC PHYSICAL DISINFECTION **METHODS**

The earliest physical method is heating, which is currently mainly used to disinfect and sterilize medical instruments and special-purpose equipment, such as sterilizing spacecraft sent to other planets. Limitations of the method are associated with the thermal stability of the processed samples or with their large size. Hard gamma radiation is also used for the same purposes, but this method has even more limitations. Various physical methods are currently known and tested for disinfection of air and water: filtration, ozonation, exposure to ultraviolet radiation [1], photocatalysis, and cold plasma. Recently, it has been proposed for the disinfection of air and water to use strong electric fields, in which destruction or electroporation of microorganisms occurs. Apart from these methods, special coatings are also being developed for disinfecting surfaces. Let us analyze these methods and equipment based on them.

3. FILTRATION

Clean air is an environment that does not support the reproduction of microorganisms; this is due to lack of nutrients and lack of moisture. In addition, the bactericidal effect of UV rays from the sun is more pronounced in the air. The viability of microorganisms in the air is provided by suspended particles of water, mucus, dust and soil fragments. Currently, the generally accepted point of view is that microorganisms in the air of closed spaces are in the form of a bacterial aerosol—a colloidal system consisting of a gaseous medium (air), in which there are tiny droplets of liquid or particles of solid matter, with the infectious material (microorganisms) confined in them.

Bacterial aerosol consists of three rather clearly separated phases, namely: solid particles, coarsedroplet phase, containing large infected droplets of saliva or mucus larger than 100 μm, and dropletnuclear phase with a size of less than 100 μm. The main amount of dangerous microorganisms comes into the air from humans, animals and products of their vital functions. Pathogenic microorganisms are released into the air together with the environment in which they are located. (For example, up to 800 particles per minute are released when talking and on average 40000 particles per minute are released at a single sneeze.) This is also true of pathogenic microorganisms that come into the air from sick people and other bacilli carriers.

When air is purified, it is more or less disinfected. When particles are removed physically from the volume by various methods, e.g., by filtration, or by oxidizing chemically harmful components in the bulk and on the surface, or by sorbing certain impurities, the concentration of microorganisms in the air mixture is always lowered. In this sense, ventilation (natural or artificial) is one of the methods for cleaning and disinfecting indoor air.

Air disinfection by filtration can reduce the concentration of microorganisms in a room to an acceptable level. This is a fairly simple and effective method for certain conditions. Two main methods are used to remove fine dust particles from the air. The first method is when cleaning is carried out using a fibrous or porous material placed across the air flow (socalled mechanical filters), and the second method is, when particles are captured by an electric field, followed by their deposition (electrostatic precipitator). For example, a highly efficient mechanical filter (HEPA filter) is designed to capture particles with a size of 2 μm or less. The filtering medium of such a filter is made of glass fibers with diameters in the range of 0.1–10 μm, and the distance between the fibers, as a rule, is much larger than the sizes of the captured particles. During air filtration, microorganisms are captured by the filter fibers and held on their surface by surface forces, in particular, van der Waals forces.

Filter systems, fillers and filtration methods are continually being improved. New filters using nanometer-size filtering polymer fibers are capable of capturing dust particles and microorganisms with sizes less than 1 μm. Such filters are expensive, have a small resource, produce great resistance to the passing air flow, but they make it possible to carry out fine filtration.

Electrostatic precipitators have been widely used for dust removal in industrial processes for over a century. In electrostatic precipitators, charged microparticles in an electric field are attracted to an electrode of a different sign (the so-called precipitation electrode). Charging of microparticles can occur as a result of friction against air in the electric field or using an additional device. Filters with the electrostatic charge on the filter itself or on particles have low cost but also low efficiency. Two-stage electrostatic precipitators contain a particle charging cascade, usually by the corona discharge, and a deposition cascade. The electrodes in the deposition cascade can be in the form of metal plates arranged parallel to the flow, with the electric field between the plates directed perpendicular to the air flow. Charged particles in the electric field move perpendicular to the air flow and are deposited on the plates. As dust accumulates, the collecting plates should be cleaned or dust shaken off.

In other designs, the collection cascade consists of several porous electrodes, to which the voltage is applied, with porous insulating plates, e.g., polyurethane foam, placed between them. All these plates are located across the air flow and cover the entire section. In this case, the captured microparticles are located in the depth of porous electrodes or porous dielectric plates, and as a rule, such plates are not cleaned, but they are replaced. The porous plates located across the flow lead to a significant pressure drop and, as a consequence, to an increase in the fan power. When such filters are mounted in the existing ventilation system, the pressure loss can be so significant that such an arrangement of collecting plates has to be abandoned.

Since air filtration will always be carried out, the filtering systems are constantly being improved. New filtration methods are also emerging, e.g., filtration by dynamic electric fields produced by special traps, which, unlike conventional electrostatic filters, can be configured to selectively remove particles that are usually poorly removed by other methods, e.g., in the range from 0.1 up to 1 μm [5, 6]. Such filters can be used not only for the selective removal of dust particles, but also for the selective removal of microorganisms of a certain size, which can significantly increase the efficiency of disinfection of the required pathogens.

4. INACTIVATION OF MICROORGANISMS IN FILTERS

In conventional filters, the captured microorganisms do not die, but remain viable for some time, i.e., the filter actually accumulates viable microorganisms during operation. Because of this, there is a risk of socalled "salvo emissions" of viable microorganisms into the air duct and then into a room. This is due to the fact that if for some reason the ventilation system was turned off and the filters were not replaced with new ones, then the next time the ventilation is turned on, the filters will be subjected to a pneumatic shock. This will cause dust and microorganisms to escape from the filter into the air duct or room. This raises a number of difficulties when changing filters, since pathogens can accumulate on them and they represent a potential hazard. Replacing such filters significantly increases the risk of contamination of the air channel and rooms with pathogenic microorganisms, as well as the likelihood of personnel contamination.

The problems of changing filters and salvo emissions are controlled risks. This means that when all the necessary requirements are met (e.g., the exact execution of the filter replacement procedure), the likelihood of negative consequences is reduced to an acceptable minimum level. However, there are also uncontrollable risks during the operation of high-efficiency particulate air (HEPA) filters. The most dangerous of them is the possibility of the growth and reproduction of microorganisms on the surface of the filters. According to the data of [7], about 20% of the filters in use grow various types of fungi (visually, the filters become overgrown with mold). Thus, the use of air disinfection systems based only on filtration in medical organizations is potentially dangerous; therefore, the use of filters with accumulation of pathogens in infectious wards is prohibited.

Some modern air filtration plants also provide additional inactivation of microorganisms in order to increase safety and efficiency. Usually there is air filtration at the first stage, and then the actual inactivation of the microorganisms trapped by the filter. Such devices can be divided into three groups:

—HEPA filters with biocidal impregnation, on which microorganisms are inactivated upon their contact with chemical compounds;

—facilities with the so-called, active filtration, in which chemically active substances (ozone, reactive forms of oxygen, radicals), which are produced by an additional device (e.g., an ozonizer or an electric discharge), are used to inactivate microorganisms trapped on filters;

—facilities in which microorganisms trapped by the filter are inactivated by ultraviolet irradiation.

However, for systems using chemical compounds (biocidal impregnation, ozone, etc.), there is a danger of the formation of forms of microorganisms resistant to the use of this chemical disinfectant. In other words, the chemical impregnation should be renewed. In addition, such facilities, especially those generating ozone, are potentially hazardous during operation, since they emit toxic compounds and require special safety measures.

Due to the aforementioned disadvantages of filtration, other methods and devices are additionally used for air disinfection, which inactivate or destroy microorganisms. Such methods include exposure to ultraviolet (UV) radiation, photocatalysis, plasma of electric discharges, etc.

5. EXPOSURE TO ULTRAVIOLET RADIATION

Inactivation of microorganisms by UV radiation has long been a generally recognized high-efficiency physical method [1, 8–10]. Open irradiators are often used for disinfection with UV radiation, since the efficiency of using the bactericidal flow of ultraviolet radiation from the lamp in this case is the highest. At present, there is a tendency to use more and more powerful UV irradiators, which provide high radiation doses in a short processing time, for the disinfection of air and surfaces [10]. The unit capacities of such systems range from hundreds of watts to several kilowatts. Similar modern mobile UV complexes are being developed in Russia and abroad [10]. Many years of practical experience in the use of UV radiation for disinfection is mainly based on the use of lamps with an electric discharge in low-pressure mercury vapor, which emit one line with a wavelength of 254 nm in the bactericidal region. Recently, major advances have been made in the development of a new generation of low-pressure UV lamps, in which the amalgam is the source of the mercury vapor. Amalgam lamps have a high linear power of bactericidal radiation, high efficiency (30– 40%) and a long useful service life (12000–16000 h).

UV disinfection using low-pressure amalgam and mercury lamps is an environmentally friendly, economical and convenient method that combines high disinfection efficiency, no harmful effects on the air, low operating costs, and simplicity of operation and compactness of UV facilities. UV radiation sources can be used for treating the air in the entire room, and in closed air ducts or recirculators. The UV radiation treatment of surfaces is also an effective method, however, unlike air, the UV radiation dose can strongly depend on the type and condition of the surface, since microorganisms can be protected by biological constituents, e.g., mucus, as a result of which in most cases the required dose of the UV radiation energy can increase significantly [1].

With regard to water disinfection, the UV technology has reached such a high level over the past 20 years that it is almost always the main one. At present, all over the world, the technology of chlorination of drinking and waste water is being replaced by the technology of UV disinfection, and ozonization in many cases is a preliminary stage before the stage of UV disinfection. Recently, there have been proposals [11–14] to use pulsed xenon lamps with a peak pulse power of 5–10 MW for disinfection, the radiation spectrum of which contains a significant fraction of UV radiation. Since the peak power of a radiation pulse from a xenon lamp can be 3–10 MW, a natural question arises whether there are differences in the germicidal treatment of media with UV radiation from such a flash lamp and conventional mercury lamps. The wide spectrum of a pulsed discharge also raises the question of whether the disinfection is affected by pulsed radiation of the visible range. To date, it has been established that pulsed radiation has a bactericidal effect, and that the mechanism of its effect on microorganisms depends on the peak power density of UV radiation, and each type of microorganism has its own value for the threshold peak power. According to the data $[11-14]$, the mechanism of disinfection by pulsed radiation has two components: one of them is the wellknown effect of bactericidal UV radiation, the other is the destruction of a microorganism as a result of its overheating when absorbing all UV radiation. When the intensity of the UV radiation pulse in the spectral A, B, C ranges (200–400 nm) is higher than 1– 3 kW/cm², microorganisms overheat and their thermal destruction occurs, since the rate of supply of radiant energy exceeds the discharge rate of the thermal energy by a microorganism into the environment [11]. It has been shown experimentally that radiation from the visible and infrared regions of the spectrum does not make a significant contribution to the heating of microorganisms [11, 12].

In accordance with the presented studies, the treatment with pulsed UV radiation can be conditionally divided into two ranges: (1) if the irradiation is much lower than the threshold value of 1 kW/cm², then this is the low-irradiation range, where only the traditional mechanism of the destruction of DNA molecules works; (2) at irradiation above $5-10$ kW/cm², the microorganism is overheated and its thermal destruction occurs. Such high irradiation can be produced by pulsed xenon lamps at distances of no more than 10– 50 cm from the treated surfaces, therefore it is already used for disinfection of medical drugs, solutions and instruments, food products, packaging materials and various surfaces for food, medical and perfumery industry. Under the indicated conditional division, naturally, there is also an intermediate region of pulse irradiation. In this region, thermal destruction does not occur, however, if the power is still high enough, then the absorption of UV radiation by the outer membranes of protein cells can ultimately damage biological membranes and disrupt the synthesis of various components of membranes and cell membranes, and then to the cell death [11]. In addition to the damage to DNA and RNA, UV radiation also induces photochemical reactions in proteins, enzymes and other molecules within the cell. Protein absorption has a local maximum of about 270–280 nm, and the protein absorption cross section increases at wavelengths below 240 nm, while there is also some absorption by the peptide bond (−CONH−) of proteins. Other unsaturated biological molecules may also be susceptible to the UV exposure. All these factors contribute to the increase in the efficiency of pulsed UV radiation. A significant disadvantage of using pulsed radiation in this pulse power range is the lack of experimental data that would make it possible to identify the mechanisms of action and determine the criteria for their implementation, as well as a large uncertainty in the effectiveness of action on microorganisms of various types.

When the pulse power in the disinfection zone is below the threshold, pulsed UV sources can be used in the same way as for conventional bactericidal lamps. In this case, the bactericidal effect depends on the fraction of UV radiation in the bactericidal range, taking into account the bactericidal efficiency [1]. In [15], the efficiency of disinfection of the Tru-D facility was compared with that of traditional low-pressure mercury lamps and equipment with a pulsed xenon lamp. Due to the fact that the power consumption and design features of the facilities are different, they were tested according to the declared passport parameters: irradiation time and operating conditions. Under the same conditions, the efficiency of UV irradiators with a pulsed xenon lamp and continuous UV-C irradiation at a distance of 4 feet (122 cm) and an exposure time of 10 min was studied. The inactivation of *Clostridium difficile* spores, methicillin-resistant *Staphylococcus aureus* (MRSA), and vancomycin-resistant *Enterococcus* (VRE) deposited on a glass slide was studied, and the effect of the concentration of pathogenic microorganisms, distance from the device, additional organic load, and also the effectiveness of the destruction of pathogens while protecting the slide from a direct radiation source. As it turned out, constant UV-C radiation made it possible to achieve the higher decrease in the initial the number of microorganisms on the slides than when using pulsed radiation from a xenon lamp. The efficiency of disinfection by constant UV-C radiation was higher than that of a pulsed emitter: for *Clostridium difficile* spores—three times, for MRSA—by an order of magnitude, and for VRE—by three orders of magnitude. The analysis of these results (under the assumption that the efficiency of the generation of bactericidal UV radiation by a xenon lamp is 10–15%, which is confirmed by numerous studies and certificates for xenon lamps produced by world manufacturers, and the energy consumption of the Tru-D facility is approximately twice more) leads to the conclusion that the obtained differences in the efficiency of disinfection are fully explained by the traditional mechanism of the effect of UV radiation on DNA.

In the case of using pulsed UV radiation for disinfecting air and surfaces in rooms, i.e., under conditions of low irradiation levels at long distances, xenon lamps have no advantages over traditional low-pressure mercury and amalgam lamps [16]. If, according to the operating conditions, the equipment without mercury is strictly required, then the pulse equipment can provide the necessary bactericidal efficiency of disinfection, but its cost is high with a relatively low resource. The final choice of the equipment should be determined by the specific task and economic feasibility.

6. PHOTOCATALYSIS

A free electron and an electron vacancy—a hole are produced upon absorption of UV radiation in a semiconductor. An electron and a hole on the surface of a semiconductor can react with molecules adsorbed on the surface, e.g., with organic molecules, which can be harmful impurities in water or air. In addition, electrons on the surface form extremely active oxidants from water and oxygen molecules, such as the oxygen ion O–, OH• radical, hydrogen peroxide and a reducing agent—the hydrogen radical. The holes have an extremely high positive oxidation potential and can oxidize almost all chemical products. The hole reacts either with water or with any adsorbed organic (in some cases inorganic) compound by oxidizing it. OH or O radicals are also capable of oxidizing any organic compound and many inorganic compounds, as well as microorganisms [17, 18]. As a photocatalyst, titanium dioxide $TiO₂$ nanoparticles in the crystalline form of anatase are most often used. The catalysts can be used in the form of a powder from nanocrystals or in the form of thin films deposited on glass filaments, polymers or other surfaces. Light destroys organic molecules on the $TiO₂$ surface and also destructs harmful microorganisms, even those with high resistance to ultraviolet light. In addition to flow-through devices for air purification, active $TiO₂$ can also be used to cover the walls of premises [18]. In this case, the entire surface of the room works as an air purifier when illuminated by solar radiation. Photocatalytic filters are quite often used as an additional stage of cleaning in air conditioning in individual premises.

Despite the possibility of the decomposition of almost any impurity, photocatalysis has not yet attracted consumers for the large-scale application. The main reasons are the low efficiency of using UV radiation and deactivation of the photocatalyst. Deactivation is associated with the blocking of active centers on the surface by other substances present in real conditions, e.g., the presence of sulfur can block the processes of disinfection during photocatalysis. Another reason is the contamination of the semiconductor either with ordinary dirt, or during the precipitation of salts or other inorganic substances. The surface of the catalyst can also be "poisoned" or covered with decomposition products, which should be removed, since in this case the processed substances and UV radiation cannot reach the active surface of the catalyst.

However, photocatalytic methods will evolve because their attractiveness lies in the fact that no other chemicals are required to destroy any impurities, and in the case of the use of solar radiation, no UV radiation sources or other equipment are required, and non-toxic substances are always the end products of the decomposition.

7. OZONATION

Ozonation by its nature is formally a chemical method, since it uses ozone, a strong oxidizing agent. But due to the fact that ozone is an unstable compound, it should be synthesized directly at the object of disinfection using an electric discharge. Therefore, this method can also be attributed to physical methods. Ozone is a poisonous substance, therefore, ozonation of premises is possible only in the absence of people or this method is used in emergency cases or in the case of severe contamination of the air and surfaces. Unlike other disinfecting substances, ozone does not form toxic products and quickly decomposes, therefore, when using it, no additional room processing is required. The use of ozonizers for the disinfection of air and surfaces gives good results in microbiology. However, it should be borne in mind that in this

case, the required concentration of ozone is many times higher than the MAC in the atmospheric air (0.03 mg/m^3) . This imposes additional restrictions on the methods of application of such treatment, moreover, the presence of excess ozone can lead to the formation of formaldehydes in the environment.

8. PLASMA AND ELECTRIC DISCHARGES

A large number of various highly active substances (electrons, ions, OH, H, O, ozone, NO, excited atoms and molecules, etc.) are formed in the plasma of an electric discharge in the air, which purify the air from impurities and perform disinfection. Corona, highfrequency and microwave discharges, as well as pulsed streamer discharge are used to clean the air. It should be noted that an electric field cleaning section with a corona discharge is used in many modern air filtration systems, in addition to mechanical filters. For disinfection and plasma medicine [19, 20], a barrier discharge with a dielectric near a high-voltage electrode is mainly used [21] or plasma jets [22] produced by discharges of different types.

In most cases, a flow of contaminated air passes through the discharge zone. Cold plasma jets are used to affect various surfaces, including when treating wounds in plasma medicine [23, 24]. Systematic studies of the effects of plasma on various microorganisms have not been carried out, but a comparative analysis of different studies can be done. Depending on the discharge and the method of its application, disinfection takes time from several seconds to several minutes. The efficiency of disinfection of air and surfaces in the discharge zone is higher than when exposed to plasma jets [21]. Perhaps this is due to the fact that in a cold plasma jet the main mechanism of destruction and death of microorganisms is the chemical action of OH radicals, ozone and other active particles, and in the discharge zone, microorganisms are additionally affected by strong electric fields, ions, electrons and electric charges produced on the surface of microorganisms in plasma. Since there are many operating factors in the discharge and the mechanisms of their action are different, a large number of studies have been devoted to determining their role, e.g., [25–27]. The least studied mechanisms are those of the action of strong electric fields and electric charge on the surface of a microorganism. In [27], the mechanism of rupture of a microorganism by an electric field was considered by the example of the gram-negative bacteria *E. coli* and gram-positive *B. subtilis*. It has been shown that due to the different structures of the membranes and the different geometric shapes of these microorganisms (*E. coli* has a small tip, which is easier to break), the required potential value on the surface *B. subtilis* should be seven times higher. In addition to the rupture of the microorganism, the process of electroporation is possible, i.e., the formation of

nanopores in the membrane, which can also lead to the death of the bacteria.

Since the effect of electric discharges is multifactorial, it is difficult to compare the effectiveness of the effect of a discharge on various microorganisms with the effectiveness of exposure to UV radiation or chemicals. However, given that the inactivation of microorganisms on surfaces by UV radiation is not as effective as for air, it can be concluded that the use of discharges for disinfecting surfaces is promising and the number of such studies will increase. It is possible that the most effective way to disinfect surfaces is the combined use of electric discharges and UV radiation, since the microorganisms that are easily inactivated by UV radiation can be resistant to plasma and vice versa.

At present, air disinfection by electric discharges cannot compete with UV radiation. There are several reasons for this, starting with the fact that the doses for most pathogens are known for UV radiation and there are methods for its application, UV lamps have a higher resource; when using electric discharges, it is necessary to take measures to remove ozone or other undesirable substances, which can be formed in the plasma.

The use of pulsed electric discharges for water disinfection is extremely limited. The disinfection mechanism is due to the appearance of a shock wave in the water by a pulsed electric discharge, the formation of OH radicals and other active substances in the water, which destroy microorganisms. In water treated with pulsed electric discharges, microorganisms do not develop for a long time, since ions of the electrode material (copper or iron), which inhibit microorganisms, pass into the water from the electrode.

Disinfection of water by pulsed electric discharges is an energy-consuming process in comparison with UV radiation: metal ions are formed in the water, the electrodes and the disinfection chamber are destroyed, and the resource of the spark gaps is limited. Therefore, the electric-discharge method of disinfection is used either in extreme situations, when it is necessary to urgently prepare drinking water in case of poor preliminary treatment, or in cases where UV radiation is not applicable, e.g., for disinfection of juices, syrups or other opaque liquids.

9. ELECTROPORATION IN AN ELECTRIC FIELD

Electroporation is the formation of nanopores in the cell membrane when exposed to an electric field. This phenomenon is widely used in biotechnology for the introduction of macromolecules into cells through the produced nanopores, e.g., DNA and RNA, into mammalian, bacterial or plant cells.

The potential of about 1 V on the membrane is required to produce nanosized pores [28–31]. It is easier to produce such a potential when the cell is in a conductive aqueous solution or in water; therefore, the electroporation process in biotechnology is carried out when exposed to high-voltage pulses (several kilovolts) in cells with water at interelectrode distances of 1– 2 mm. If the exposure to the electric field lasts long enough, then the pore sizes increase, and ion and molecular exchange between the cell fluid and aqueous solution begins, as a result of which cell destruction can occur. The experiments have shown that when exposed to pulses of an electric field with a strength of 30 kV/cm and a duration of 500 ns, microorganisms (except for spores) of the species *Aspergillus niger*, *Saccharomyces cerevisiae*, *Bacillus cereus*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* decrease by 3–4 orders of magnitude after 300 pulses [32]. However, in [33], no effect of the destruction was found when *Bacillus Stearothermophilus* in water was exposed to the field strength of up to 40 kV/cm; therefore, the studies were carried out at the field strength of up to 180 kV/cm.

Thus, for biotechnology or medical purposes, electric fields of several kV/cm are sufficient, but for disinfection or sterilization, the electric field strength should be an order of magnitude higher. This method was tested for water disinfection [34] with foamed silver electrodes; however, it has not yet been used, since the used silver electrodes dissolve when current passes that leads to their destruction and contamination of water with silver ions.

Higher electric field strengths of 20–100 kV/cm are required for the electroporation process in air. Higher electric field strengths in air are explained by the fact that the potential on the membrane in water is produced due to both the flow of the electric current and strengthening of the electric field in the membrane with respect to the field in water, since the permittivity of water (about 80 at room temperature) is much higher than that of the membrane (about 7); therefore, the field strength in the membrane increases with respect to the external electric field strength. On the other hand, the air conductivity is zero, the relative permittivity is 1, therefore, the external electric field inside the membrane weakens, and it is necessary to increase the external electric field strength in order to produce the critical potential on the membrane. However, the air breakdown occurs at the electric field strength higher than 31 kV/cm, the electric discharge and plasma are formed, and the electric field strength decreases. Therefore, the high electric field strength can be produced only for a short time in the pulsed mode, e.g., in the corona discharge mode or without the discharge, but at lower values of the electric field strength. It should also be noted that the smaller the size of the microorganism, the higher the electric field strength should be, since the field is displaced from the cell onto the membrane. Accordingly, the larger the size of the microorganism, the higher the potential appears on the membrane, while the potential difference across the entire microorganism is comparable to the potential difference across the membrane. Therefore, the field strength for electroporation should be higher for viruses, the size of which is smaller than that of bacteria, and, accordingly, the method should be less effective.

The exchange with the external environment is necessary for the destruction of a microorganism after the production of pores. In the case of water, ionic and molecular exchange occurs, but in the case of air, the surface tension prevents the outflow of intracellular fluid, so the pore size should be larger. Most likely, the electric field strength should be so high for the destruction of microorganisms in the air that the microorganism is broken as it was considered in [34], and not just the nanopores are formed.

Thus, the method of electroporation for disinfection works well enough in water, but its application for air is associated with great technical and fundamental physical difficulties in producing strong electric fields. Nevertheless, in the opinion of the authors of works [35–37], the equipment developed by them operates using the method of inactivation of microorganisms in the air by constant electric fields.

According to [35–37] and materials on the manufacturer's website, this air disinfection system functions as follows: corona discharges with different polarities are produced in the inactivation zone, and microorganisms are repeatedly exposed to constant electric fields with sharply changing strengths and gradients, as well as to ions of opposite signs, as a result of which irreversible damage or complete destruction of microbial cells occurs. In the fine filtration zone, debris of destroyed cells and other particles are captured on highly porous nanoelectrodes of an electrostatic precipitator with a high dust capacity. At the same time, the filtration efficiency corresponds to filters of a class not lower than H11. A super-long standard service life of at least 10 years is declared.

We analyze these works and this equipment for air disinfection. Structurally, the analyzed system consists of several conventional mechanical filters as well as electrostatic filters in which foamed metal (titanium or nickel) plates with the PPI30 porosity (precipitators) located across the air flow are used as precipitation plates. The plates of 1-cm-thick reticulated polyurethane foam with the PPI 20 porosity, which serve simultaneously as insulators and filters, are placed between the metal plates. The particles in air are charged in corona discharges generated in metal cylinders. In this case, a metal needle, which is a high-voltage corona discharge electrode, is located along the axis inside each cylinder. The supply voltage of the corona discharge and between the metal plates is 4.5 kV. The design is made non-separable, so it is impossible to change or clean the filter elements.

We consider the possible mechanisms of inactivation of microorganisms in this system.

The corona discharge zone is small compared to the cross section region, so a small fraction of microorganisms passes through the active plasma. The diameter of the needle with the corona discharge is 0.35 mm, therefore, over time, the needle is destroyed and the structure becomes dusty, as a result of which the corona discharge ceases to burn steadily. A time of several minutes is required for inactivation in the plasma outside the corona discharge zone; this is well known from works on plasma medicine, which have been actively carried out over the past 10–15 years. Consequently, the corona discharge section is not able to effectively disinfect the blown air. If it were effective, then corona discharge would have been used a hundred years ago.

9.2. The Electric Charge on the Surface of Microorganisms

This mechanism can work but its effectiveness can differ for different types of microorganisms by several orders of magnitude. High electric fields are required for the guaranteed charging of microparticles in the corona discharge, however, at a distance of $1-2$ cm from the high-voltage needle electrode, the electric field strength decreases by 20–50 times, and the concentration of ions at the declared power of the facility in these regions is also small. For these reasons, this mechanism will be ineffective.

9.3. Exposure to the Electric Field

When exposed to a high electric field, the destruction of the membrane of microorganisms or puncture of membranes (electroporation) can occur. As mentioned above, the electric fields of 40–150 kV/cm are required for electroporation in air that cannot be produced in air, since the electrical breakdown occurs first. In this system, the field strength reaches the required value near the 0.35-mm-diameter corona electrode but only at very small distances from the electrode, and the field decreases by a factor of 10 at the distance of 1 cm. In their patents, the authors propose to fabricate bumps with a diameter of 30 μm or less (actually needles) on the surface of the plates in order to enhance the electric field near the deposition metal plates from 5 to 100 kV/cm or higher. However, the electric field near such points decreases rapidly over distances on the order of their diameter, so only a small fraction of microorganisms can pass through the region of the enhanced field. It should be especially noted that the electric field inside the foamed metal plates is small due to the laws of electrostatics, and the field enhancement inside the polyurethane plates is also too small, since the volume of the dielectric is less than the volume of the pores. The destruction of the shell of microorganisms in the electric field depends on the shape of bacteria or spores, e.g., gram-negative bacteria *E. coli* is destroyed well in the electric field, and the destruction of gram-positive *Bacillus subtilis* spores is several orders of magnitude less (the difference may be five orders of magnitude) [27].

The dust in the air settles well near these points, therefore, the micropoints will be covered with dust after a short time, and microorganisms will not get into the region of the high field. For these reasons, the electroporation mechanism in this design works extremely inefficiently.

9.4. Inactivation on the Surface of the Filters

Microorganisms deposited on the surface of the filters can be destructed due to low doses of ozone, active particles from the corona discharge, and catalytic properties of the surface of collecting metal plates. This mechanism seems to be the most probable, but its efficiency sharply decreases when the surface is dusty.

9.5. The Effect of Collected Dust

The design of this system is made non-separable, so it is impossible to change or clean the filter elements. The average concentration of dust in the atmospheric air is 0.15 mg/m³, and that of up to 0.5 mg/m³ is allowed in places of mass gathering of people. Based on the dust holding capacity of the used filter materials (the dust holding capacity of the reticulated polyurethane foam is 156 g/m^2), the filters will be filled in 400–500 h of the continuous operation provided that 50% of the dust settles on the pre-filter, at the declared filtration efficiency of the cleaning class H14 $(99.995%)$ and the air dust content of 0.5 mg/m³. More than 5 kg of dust should accumulate in the facility after ten years at the capacity of $130 \text{ m}^3/\text{h}$ and operation of 8000 h per year. The facility cannot effectively inactivate microorganisms with such an amount of dust, and the accumulated dust together with microorganisms will be periodically thrown into the room. It should be noted that when a layer of dust is accumulated on the collecting electrodes, the electric charge is accumulated on it. The latter will initiate periodic breakdowns of this layer of dust that can lead to the ignition of the dust and the entire device.

Consequently, this facility is actually a good filtering system. Therefore, the efficiency of removing microorganisms from the air is high only on a new facility, while the efficiency of electroporation is always low. Note that the disinfection efficiency drops sharply as dusting increases. In other words, the resource declared by the manufacturers is unrealistic.

Thus, the electric field strengths should significantly exceed the values, at which the electrical breakdown of air occurs, in order to inactivate of microorganisms in the air by electroporation or to destroy them. Therefore, it is impossible to produce the required electric field in the static case. High electric fields in air can be obtained only by using short electric pulses with a duration of tens or hundreds of nanoseconds. Such pulse equipment has a small resource, it is expensive, its operation requires highly qualified personnel, and there are difficulties with the electromagnetic shielding of such equipment for the safe operation of computer equipment and personnel. In addition, there is still no sufficient database on the required electric field strength to inactivate all pathogens.

10. FABRICATION OF SPECIAL COATINGS

Microorganisms can persist for a long time and also develop on surfaces, especially at high humidity. For example, fungi and mold can actively multiply on plastics, metals, alloys, rubber, and ceramics. As a result of the vital activity of such microorganisms, the irreversible destruction of the surface occurs, and the spores of microorganisms and harmful substances can spread by air flows to non-contaminated surfaces. The chemical treatment or disinfection of surfaces is most effective, but its use is limited or impossible in closed spaces where people are present. The treatment with ultraviolet radiation or plasma of an electric discharge is not possible for closed surfaces, and it requires special sources in the case of surfaces with complex shapes.

A promising direction is the fabrication of structural materials or coatings that can exhibit their own biological activity towards certain microorganisms, as a result of which microorganisms on such a surface either are destructed or cannot actively multiply. Such coatings can be fabricated based on linear-chain carbon in the SP1 hybridization. It is necessary to learn how to fabricate biologically active layers on various classes of structural materials and polymers in order to apply these results widely.

At present, a technology has been developed for the condensation of carbon vapors on the surface for the deposition of a film of two-dimensionally ordered linear-chain carbon in the form of linear carbon chains in the perpendicular orientation to the surface. The studies in air at high humidity have shown that such carbon materials exhibit the high antimicrobial activity against gram-positive microorganisms of the *Staphylococcus aureus* species and gram-negative microorganisms of the *Pseudomonas aeruginosa* species [38], and also inhibit the growth of various fungi species. The studied carbon coatings are safe for humans and can be applied in places where people stay for a long time and in closed spaces.

11. CONCLUSIONS

Currently, a number of different physical methods can be used for effective disinfection. UV technology is still the most effective, safe and economical for

water disinfection. The problem of air disinfection in crowded places is becoming more and more urgent, since airborne infections can be spread over several hours or days by passengers of airplanes and highspeed trains in most developed countries. As a result of this threat, the known methods of air disinfection are being improved and new ones appear. The most effective is a multi-barrier system for air purification and disinfection, which includes both filter systems and systems for inactivation of microorganisms. Over the past decade, the power and reliability of low-pressure germicidal amalgam UV lamps have significantly increased, so the method of air disinfection with UV radiation is a reliable, simple and effective method. Electric discharges for air disinfection are still rarely used, and the use of electric discharges seems promising for surface disinfection and plasma medicine, which is confirmed by numerous studies all over the world. The method of inactivation of microorganisms in air only by electric fields is associated with technical and fundamental difficulties in producing strong pulsed fields, therefore, it is unlikely that competitive industrial facilities will be developed in the near future.

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