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ENDOTOXINS IN THE AIR OF OBJECTS OF WASTE AND WASTEWATER MANAGEMENT INFRASTRUCTURE. REVIEW OF THE APPLIED METHODOLOGY AND OBTAINED RESULTS OF THE STUDY

The paper analyses the variability of the methodology of collecting samples of endotoxins from the air in the premises and the vicinity of objects of waste management and wastewater treatment infrastructure, methods of their preparation, determination of endotoxin content, and results of research to date. The high sensitivity of analytical methods enables the analysis of the concentration of endotoxins in air samples with a small volume. After freezing, they can be stored for a long time. The effectiveness of extraction of endotoxins from bioaerosol samples can be improved by adding Tween 20 or Tween 80 to water. So far, factors determining the variability of concentrations of endotoxins in the air in the premises and the vicinity of objects of waste management infrastructure were determined. Further research in the scope is necessary. This will allow the determination of acceptable levels of endotoxin in the future. The impact of endotoxins on human health and the specificity of sewage and waste management must also be taken into account.

1. INTRODUCTION

Air (atmospheric and indoor) is not the environment of the constant presence of microorganisms due to the lack of nutrients and unfavorable physicochemical conditions. It is, however, a route of transmission to environments favoring the growth and reproduction of viruses, bacteria, microscopic fungi, and algae, as well as plant pollen. The air also carries products of the metabolism of microorganisms such as endotoxins and mycotoxins [1]. The dust of organic origin floating in the air is described by the general name of bioaerosol.

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Some of the microorganisms floating in the air are pathogenic or potentially pathogenic for people and bred animals. Other cause phenomena unfavorable for the economy (e.g., biological corrosion of construction materials, rotting of food). Preventing these phenomena requires among others monitoring the concentration of bioaerosol in the air. The determination of the concentration of all the occurring microorganisms in the air is practically impossible due to their species diversity and undetectability of part of them utilizing culture methods. Therefore, monitoring of the sanitary state of air is limited to the determination of the concentration of selected groups of indicator microorganisms. One of the indicators of the sanitary state of air is the concentration of endotoxins – toxins constituting lipopolysaccharide complexes included in the composition of the cell wall of gram-negative bacteria. Results of research conducted to date show that persons employed in waste management and wastewater treatment are exposed to the effect of endotoxins present in the air in relatively high concentrations, reaching even a dozen of million EU/m^3 [2].

No uniform research methodology has been developed and commonly adopted so far regarding concentrations of endotoxins in the air. It concerns collecting samples, their storage, extraction, the technique of determination of the content of endotoxins in samples, and determination of acceptable concentrations. Therefore, the objectives of the presented paper were as follows:

- analysis of the applied methodologies of sample collection and their preparation for analysis, and proposing a uniform methodology,
- analysis of the current state of knowledge on the emission of endotoxins to atmospheric air by objects of waste management and wastewater treatment infrastructure.

2. RESEARCH METHODOLOGY

2.1. SAMPLE COLLECTING

Owing to the high sensitivity of methods of quantitative determination of endotoxins, it is possible to determine their content in small air samples of even 0.666 dm^3 [3]. Small sample volumes allow a considerable reduction of sampling duration, even to 9 min [4]. This enables the determination of concentrations related to short-term events, e.g., waste unloading. Results of the analysis of samples collected over a period of several hours allow the determination of the mean concentration of endotoxins in the air, e.g., during one work shift, with no necessity of analysis of many samples and averaging the results [5, 6]. Sample collecting with breaks adjusted to the time of breaks of employees is also often applied [7].

Small bioaerosol samples can be collected using pumps with a low flow rate – from 1.0 dm^3/min [8]. The application of pumps with a higher flow rate is not excluded, e.g., 12.5 dm^3/min [9].

The application of stationary samplers [4, 6] enables the analysis of the concentration of endotoxins at selected study sites, located, e.g., at a specified distance from the emission source, or in selected rooms or work stations. The use of personal samplers allows the determination of the exposure of an employee to endotoxins during a single shift or during performing particular tasks [10, 11].

Despite their size at a level of 30–50 nm, endotoxins are a component of bioaerosol and are usually sampled using low-flow dust monitors on filters made of glass fibers [2, 6], polycarbonate [12, 13], Teflon [14, 15] or cellulose ester [12]. The applied filters usually had a pore diameter of 0.8 μm [7, 16] or 1 μm [4], pores of 1.2 μm [17], exceptionally less, e.g., 0.4 μm [10], or more, e.g., 2 μm [16]. Some authors collected bioaerosol samples from the air utilizing scrubbers filled with 50 cm^3 of apyrogenic water [9], or 20 cm^3 physiological saline [18]. Glass filters were the most commonly applied, followed by filters made of polycarbonate, and sporadically of other materials. The filters usually had pore diameters of 0.8–1.0 μm.

The common application should be one of the criteria of selection of the sampling methodology. It enables comparison of the obtained results with results of other authors. An important criterion of selection of the sampling methodology is its effectiveness. Results of studies conducted so far showed that the effectiveness of sample collecting using scrubbers filled with 20 cm^3 of apyrogenic physiological saline is similar as in the case of sampling on filters made of polyvinyl chloride [18]. More endotoxins were retained on a glass filter than on one made of polycarbonate [13]. Filters made of cellulose ester allowed extracting twice less endotoxins than filters made of glass fiber, Teflon, and polycarbonate. No differences were recorded in the quantities of endotoxins extracted from filters made of different types of glass (borosilicate and soft) and polycarbonate [12].

Common application and proven effectiveness of filtration allow recommending the use of glass filters in further research works concerning concentrations of endotoxins in air. No experimental data are available, however, regarding the effect of pore sizes on the effectiveness of sampling air-suspended endotoxins, filters with the pore diameter of 0.8–1.0 μm are commonly applied, a size larger than endotoxins. It, therefore, remains unknown whether the application of filters with smaller pores increased the efficiency of bioaerosol sampling for the determination of concentrations of endotoxins in air.

2.2. TRANSPORT AND STORAGE OF SAMPLES

Endotoxins are persistent substances, although they can sometimes be subject to decomposition. To avoid that, the collected bioaerosol samples need to be analyzed possibly quickly, e.g., within 24 h [2], or transported and stored at a low temperature. Authors of available papers usually froze the collected samples or their extracts to –20 °C [3, 5], and more seldom to a temperature of –80 °C [6]. Others store samples at 4 °C [4, 9] or 7 °C [19]. According to Spaan et al. [20], no significant differences in the content of endotoxins occur between samples stored at a temperature of 4 °C and –20 °C. Bioaerosol samples for analysis of endotoxins in cold storage conditions can be stored over many months. Filters with aerosol samples can be frozen, as well as the extract [12, 14], or both [20]. In cold storage conditions, they can be stored for many months. The amount of endotoxins on filters stored at a temperature of –20 °C did not decrease after 2–3 years [19]. The situation was similar in the case of extract stored for 1 year at 7 °C [12]. Freezing to –20 °C, defrosting, and freezing again caused a decrease in the content of endotoxins in a sample by 20% (household bioaerosol with endotoxins), or even by 25% (pure commercial endotoxin) [12]. Data available in the literature allow recommending freezing samples to –20 °C, if they cannot be extracted and tested for endotoxin content directly after sampling.

2.3. EXTRACTION OF ENDOTOXINS

Endotoxins collected on filters, and not for scrubbers, must be extracted to a solution. Authors of available papers dissolved them in:

- apyrogenic water [2, 7],
- apyrogenic water with an addition of 0.05% Tween 20 [1, 5] or 0.04–0.05% Tween 80 [17, 18],
- tris buffer [21],
- 50 mM potassium phosphate with 0.01% triethylamine [13].

Morgenstern et al. [19] collected bioaerosol samples to scrubbers filled with physiological saline solution. It could therefore be also used for the extraction of endotoxins from the filter. Authors of available papers usually extracted endotoxins from the filter by dissolving them in water or in water with an addition of non-ionic surfactants such as Tween 20 and Tween 80. They are surfactants that increase the efficiency of endotoxin extraction with water up to seven times [12, 21]. Authors of the majority of papers used 5 cm^3 [2, 5] or 10 cm^3 of solvent [1, 7], exceptionally different volumes, e.g., 6 cm^3 [17]. Only Thorne et al. [13] applied extraction in 30 cm^3 of apyrogenic water, and Duchaine et al. [18] in 30 cm^3 of apyrogenic water with an addition of 0.04% Tween 80.

Few authors provided information regarding temperature at which extraction was conducted. Spaan et al. [21] conducted it at room temperature, Duchaine et al. [18] at 37 °C, and Duquenne et al. [4] at 60 °C. Thorne et al. [13] compared the efficiency of extraction at 22 and 68 °C, and concluded that heating did not affect the extraction efficiency.

The most frequently applied extraction method is shaking or swishing filters in a solvent. Authors of almost all available papers applied 60 min shaking or swishing [1, 5] exceptionally shorter – 10–30 min [7, 13, 17] or longer – 120 min. [13], and even overnight [18]. Some authors provide information regarding the frequency of shaking, e.g., 25 or 160 oscillations/min [1, 20], and regarding the shaking amplitude, e.g., 15 cm [20]. A rarely applied alternative to shaking is sonication for 20 min [4] or 60 min [14]. Duquene et al. [4] also provide information regarding the frequency of applied ultrasounds (47 KHz).

Available literature data point to similar efficiency of both extraction methods, namely shaking and swishing, and sonication [2]. The reduction of the commonly applied shaking time (60 min) to 10 min also caused no decrease in the efficiency of endotoxins extraction [21]. This suggests that shaking and swishing, as well as sonication, can be applied alternatively, and the commonly applied 60 min shaking time ensures high efficiency of extraction, irrespective of the variability of shaking parameters such as frequency and its character (swishing, orbital and horizontal shaking).

2.4. DETERMINATION OF THE CONTENT OF ENDOTOXINS IN SAMPLES

Three fundamentally different methods of analysis of endotoxins have been developed so far. The oldest one involves the determination of the pyrogenic effect of endotoxins injected in experimental animals. The method has not been applied by any of the authors cited in this paper. Currently, the most commonly applied method uses the ability to produce colored products of the reaction with the participation of among others endotoxins and enzymes contained in the amebocytes of horseshoe crab (*Limulus Amebocyte Lysate* – LAL). This also concerns studies on endotoxins in atmospheric air in the premises and in the vicinity of objects related to waste and wastewater management. The method was applied by authors of all papers cited herein. In the case of studies on endotoxins sampled from the air, the most important advantage of the LAL method in comparison to the determination of the pyrogenic effect of endotoxins in experimental animals is a considerable decrease in the size of samples needed for the analysis. A further decrease in sample size is made possible by modifications of the LAL method permitting analyses in microplates and dynamic measurement of absorbance. It also enables a decrease in the amount of required reagents. The abundance of horseshoe crabs is limited. They are captured for blood collection and then released into the sea. The demand for reagents produced from horseshoe crab blood is high. Medicines and numerous medicinal materials must be free from endotoxins. This requires testing them in those terms. An increase in the global demand for commercial tests permitting analysis of endotoxin content, primarily in medicines and medical materials, as well as the limited abundance of shoehorn crab, result in the necessity of search for alternative methods. Therefore, the method of quantitative determination of endotoxins has been developed, employing techniques of fluorimetry or colorimetry based on reagents produced with no use of horseshoe crab blood [22]. It is currently less common than the LAL method, also in research on endotoxins contained in the air in the premises of waste and wastewater management objects. This is evidenced by the failure to apply it by authors of papers cited herein. The method shows sensitivity similar to that of LAL, and could be applied for the analysis of endotoxins contained in the air. Such applications will probably appear in the future.

3. RESULTS OF STUDIES TO DATE

Concentrations of endotoxins in atmospheric air and indoor air of objects related to waste and wastewater management show considerable variability, from tens [15] to a dozen million [2]. Results of studies to date enabled the identification of at least some of the factors determining their concentration. They include among others the concentration of suspended solids in air and the concentration of bacteria. The correlation coefficient r between the concentration of suspended solids and concentration of endotoxins to which employees of a large waste composting plant were individually exposed was 0.783, $p < 0.05$. Therefore, the concentration of suspended solids can be a good predictor of the concentration of endotoxins [1]. This conclusion also concerns the concentration of mesophilic bacteria (Gram+ and Gram–), determined by breeding methods. The coefficient of correlation between the concentration of mesophilic bacteria and concentration of endotoxins in the air of a wastewater treatment plant was in a range of 0.74–0.89, depending on the used microbiological substrate [23].

Authors of papers to date, applying different techniques of sample collection, determined the variability of concentrations of endotoxins in the air:

- in the vicinity of particular objects of a wastewater treatment plant and installations processing waste, and the function of distance from such objects [24, 25],
- to which employees at different positions are exposed [7, 21],
- to which employees are exposed during the performance of particular tasks [2, 10].

Results of these studies show exposure to the highest concentrations of endotoxins of employees working in places related to:

- wastewater movement: including its stirring, pumping wastewater from the septic car, cleaning chambers of the treatment plant or sewage system, dewatering and drying of waste [4, 21],
- waste movement: segregation, mixing, transfer of waste [1],
- anaerobic digestion and sieving of compost [25].

Concentrations of endotoxins inside the wastewater treatment plant were higher than outdoors [26], similarly as in the case of the waste collection and processing plant [25]. In cabins of vehicles working at the composting plant, the concentrations were considerably lower than outdoors. Air filters and systems providing higher than average pressure inside the cabin reduced endotoxin concentration by 93–98% in comparison to concentrations in outdoor air [25]. Employees working with the sewage system inhaled more endotoxins while performing works inside the sewage system (inspection and cleaning) than outdoors [4]. In the wastewater treatment plant analyzed by Thorn et al. [7], the level of exposure to inhaling endotoxins at particular workstations was assessed by the authors as relatively stable. Workers in wastewater plants are more exposed than workers in the wastewater net system [27]. Exposure of particular employees at the same stations in the chain of waste collection and processing analyzed by Wouters et al. [28]

showed considerable variability. Differences between particular employees at the same stations were higher than between the stations.

In the case of employees cleaning devices at the wastewater treatment plant, exposure to endotoxins depends on the applied cleaning technique. The concentration of endotoxins in the air increased when treated wastewater was used for cleaning instead of tap or surface water. Using a fire hose with a fully opened mouth brought about a decrease in endotoxins exposure in comparison to a partially obstructed mouth. The exposure was also reduced by turning on ventilation. The distance at which an employee stood from the cleaned object, or pressure of the liquid used for cleaning was of no significance [5].

Due to the variability of results of measurements of endotoxin concentrations in the air in wastewater treatment plants and waste processing plants obtained by the authors of particular papers, the exposure of employees to endotoxins was assessed by them in different ways. It was relatively low: in the case of waste collection and processing [28], including at household waste containers [29] and in a wastewater treatment plant [21]. Sykes et al. [1] assessed the exposure of employees of a waste composting plant as higher than average. The concentration of endotoxins in the air of a hospital wastewater treatment plant was considerably higher than in Danish cities [17].

Papers concerning the spread of endotoxins around wastewater treatment plants and waste management plants are scarce. Their concentration on the downwind side of the waste composting plant increased at a distance of up to 80 m, then decreased, and at a distance of 100–150 m, it unexpectedly increased again [4]. In the case of spreading bioaerosols around the wastewater treatment plant, it was determined as difficult to model, and potentially dependent on land relief and the presence of terrain obstacles such as tree stands, railway banks, or roads with intensive traffic. In the vicinity of a wastewater treatment plant (or waste management plant), additional sources of emission of bioaerosols can also occur, in the form of, e.g., eutrophicated water bodies. The area around a wastewater treatment plant or waste management plant functioning over a long time can also be subject to microbiological contamination. In that case, bioaerosol, i.e., also endotoxins, can be subject to secondary blowing away from the substrate [30].

The effects of exposure of workers in waste and wastewater management facilities to endotoxins are like the effects of exposure to other environments [31]. The effects, however, do not differ from those of exposure of people to endotoxins in other environments. In the case of recycling employees, respiratory and gastric ailments were to a greater degree related to exposure to (1-3)-β-D-glucans than endotoxins [16]. In the case of employees of the wastewater treatment plant, the factor analysis showed that the dependency on vocational exposure to endotoxins was manifested for skin conditions, symptoms in the upper and lower respiratory tract, and flu-like and systemic symptoms [26]. No literature data are available regarding the effect of endotoxins emitted by waste management and wastewater treatment plants on the health of residents of housing estates neighboring such plants. Until the preparation of such papers, it is possible to assume that it is similar to the effect analogical in terms of concentration, frequency of occurrence, and duration as in persons exposed to endotoxins in other environments.

Data on concentrations of endotoxins in the air in the premises and the vicinity of waste management objects have been so far collected in few plants. The concentrations of endotoxins in air, however, depend on many factors: the composition of waste or wastewater, the technology of their processing or treatment, or current meteorological conditions. Such plants are located in places different in terms of land relief, which affects the distribution of pollutants, including endotoxins, in the air. Caution is recommended in generalizing study results regarding concentrations of endotoxins at waste management and wastewater treatment plants about similar plants in other climatic zones. Results of studies to date are also insufficient to determine acceptable concentrations of endotoxins in the air that could be commonly adopted. So far, they have only been introduced in some countries, e.g., in the Netherlands [32]. It is therefore justified to undertake further research in the scope.

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