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NEW METHOD FOR EVALUATION OF THE MICROBIOLOGICAL QUALITY OF WATER

Determination of the microbiological quality of water is one of the central tasks of environmental engineering. Reliability and accuracy of the results depends largely on the sampling system and the methodology carried signs. Typically, they are used traditional indirect methods, breeding: plate method or test tube fermentation. In the methods, seed stocks, it is assumed that each individual cell of bacteria transferred to a solid substrate colony grows. Thus, in fact, the result of the study determines the number of units capable of forming colonies, which is not equivalent to the amount of the living. Even larger errors are subject to the results of the analysis of test tube fermentation, which can only be the most probable number of bacteria in a given volume. It is therefore only estimating the number of microorganisms. Moreover, indirect methods are extremely time consuming (about 1 - 7 days of). Flow cytometry is an analytical technique that allows for the rapid measurement of scattered light and fluorescence signals emitted by cells exposed respectively. Allows for a qualitative and quantitative assessment of the physical and biological properties of the cells in a short time. The article presents the results of research on the amount of bacterial cells in different water: surface, groundwater, rainwater and tap water using flow cytometry and compare this results obtained with traditional methods of reference. The microbial results in all the tested waters by flow cytometry are much higher than the amount of the reported conventional methods.

Keywords: water bacteriology, bacterial plate count, drinking water, flow cytometry

1. Introduction

The assessment of the microbiological quality of water is one of the overarching environmental engineering task. The reliability and accuracy of the

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results depends largely on the sampling system and methodology which carries out determinations. Traditional methods are typically used: inoculation's plaque method balanced or fermentation-specimen method. In inoculations' methods we assume that from every single cell bacteria moved permanently to the ground there is a colony. Therefore, in fact, the result of the test specifies the number of units capable of colonization, which is not equivalent to the actual number of live bacterial cells in the water. It is estimated that only 60-90% of the bacteria in the water is active and living, and the other is inactive bacteria, which, however, in favourable conditions can develop [2]. Another source pays attention to that only an average of 1% of the bacteria in water can be used to grow in traditional methods [1]. Even larger errors are burdened with the results of the analyses of fermentation-specimen methods, which can be used to specify only the most likely number of bacteria in a given volume. So this is just estimating of the amount of microorganisms. In addition, breeding methods are extremely time consuming. The preparation and implementation of one analysis time about 3 hours, and the result is obtained only after about 1-7 days. However, water safety information in terms of a microbiological test shall be assessed primarily on the basis of the results of the traditional breeding research, enabling the growth of bacteria colony on nutrients [3]. There are different methods of farming of the microorganism, where all sorts of inoculation techniques and ground. The choice of medium, which is a source of microorganisms for nutritional food, energy and the environment life depends on their nutritional requirements, as well as the purpose of the test. [7,12]. In order to ensure that the multiplication of cells, i.e. (intensive) for the development of the population, you must also enable the appropriate culture conditions [7].

Traditional methods are commonly used to assess the health status of the waters, both in Poland and in the world. For some time applied microbiology found the modern technique for flow cytometry. Diagnostic method based on flow is much faster, and more versatile [3]. It enables the characteristics of qualitative and quantitative test cells [10]. It uses physical phenomena and chemical processes so you can quickly detect and make characterization of single-cell microorganisms [9].

Flow cytometry is a technique of analysis, which allows for a quick measurement of scattered light or fluorescence signals emitted by an appropriately exposed cells. Allows for qualitative and quantitative evaluation of the physical properties and biological cells in a short period of time. In addition to measurements of fluorescence intensity measurements are distributed injected dyes fluorochromes. Pigments in the cells may occur naturally, most often they are introduced to them by previous measurements of the colouring process. To this end, the fluorochromy is combined with the appropriate matching antibodies to antigens present on the surface of cells, and then are put into suspension of complexes: dye-antibody. At the time of measuring the radiation that has not dissipated, it can be absorbed by the fluorescent dye. It raised fluorochrome, returns to the ground state energy and why it is accompanied by the emission of photons-fluorescence. The fluorescence can be detected in Green (FL1), Orange (FL2) and Red (FL3) [10]. In the microbiology of water, it is preferable to use fluorescent dye (520 nm), the intensity of the fluorescence is directly related to the content of nucleic acids in the cells to be detected, as well as green fluorescence (533 nm) [8]. This type of colours to distinguish organisms with high and low content of nucleic acid that is living, inactive [3].

The article presents the results of a study of bacterial cells in a variety of waters: surface water, groundwater, rainwater and water using flow cytometry and comparison of these values with those obtained by traditional reference methods.

2. Research methodology

To compare the amount of microorganisms the microbiological tests were carried out in the following waters:

1) surface water (Watering Place Gravel Rzeszów),
2) underground water (samples taken at different stages of the treatment process at the station in Wola Komborska),
3) tap water (a collection of Rzeszów),
4) rainwater collected on the outskirts of Rzeszów,
5) water from the indoor swimming pool in Przeworsk.

2.1.Traditional breeding methods

The number of bacteria of the heterotrophic species in 1 ml of water was described by deep-sea culture: after 24 hours of incubation at 37°c is a mesophylic and after 72 hours incubation at a temperature of 22°c – psychrophylic bacteria. In order to compare the development of micro-organisms on different media used agar nutrient reference hereinafter referred to as the agar A and the agar R (enriched).

2.2. Determination of the quantity of microorganisms by flow cytometry

To carry out the study, we used flow cytometer: *CyFlow Cube 6* and fluorescent dye: *Sybr Green I.* [8]. The results obtained with cytometer were converted to the quantity of microorganisms found in 1 ml of water before the test, taking into account the dilution used. Using flow cytometer settings when executing in study of the water flow, and voltage depending on the course of measuring (counting) of organisms in the water.

3.Test results and their discussion

The results of bacteriological tests of surface water, underground, water supply, rainwater and indoor swimming pool, respectively shows in tables 1-5.

Table 1. The results of microbiological studies of surface water

Tabela 1. Wyniki badań mikrobiologicznych wody powierzchniowej

No	**Psychrophilic bacteria [cfu/ml]**		**Mesophilic bacteria [cfu/ml]**		**Flow cytometry [counts/ml]**		
	Agar „A”	Agar „R”	Agar „A”	Agar „R”	LNA*	HNA*	Totality
1	250	540	135	370	3482600	6892300	10374900
2	2550	4300	1400	1700	5517400	7961000	13478400
3	550	1200	340	600	3519200	2706400	6225600
4	4200	6800	1800	2200	3519200	2706400	6225600
5	700	2300	1200	1580	3439300	1980000	5419300

*LNA – Low Nucleic Acid
*HNA – High Nucleic Acid

Table 2. Comparison of the results of microbiological underground water from treatment plants in Wola Komborska traditional methods of research and flow cytometry

Tabela 2. Porównanie wyników badań mikrobiologicznych wody podziemnej ze stacji uzdatniania w Woli Komborskiej metodami tradycyjnymi i cytometrią przepływową

No	**Psychrophilic bacteria [cfu/ml]**		**Mesophilic bacteria [cfu/ml]**		**Flow cytometry [counts/ml]**		
	Agar „A”	Agar „R”	Agar „A”	Agar „R”	LNA*	HNA*	Totality
1	0	6	0	16	296 517	119 075	415 592
2	1	9	1	28	285 012	172 176	457 188
3	0	4	3	14	100 236	152 487	252 723
4	2	3	7	43	77 610	25 910	103 520
5	1	14	3	21	108 196	46 833	155 029

1 - raw water, 2 - water after aeration, 3 - water after deironing, 4 - water after demanganization,
5 - contacting the water in the tank

The value of the number of bacteria derived from traditional breeding methods are always much lower than the numbers of bacteria obtained by R agar, and using flow cytometry. The total number of test mesophilic and psychrophilic bacteria uses two types of media, agar or reference ' A ' and ' R ' agar enriched by the ground. By far higher numbers of bacteria were obtained on agar "R" in the case of both mesophylic and psychrophylic in all tested samples of the water. ' R ' agar is a culture medium containing substances that allow the growth of microorganisms of the heterotrophic species of larger nutrient requirements [4]. The results of the quantity of the microorganism obtained by flow cytometry in different types of water surveyed strongly deviate from the values obtained for breeding methods. The biggest difference we can observe in the case of waters, which theoretically should be very clean bacteriologically-underground water at different stages of treatment (tab.2) and tap water (tab.3).

Table 3. The results of bacteriological tests of tap water

Tabela 3. Wyniki badań bakteriologicznych wody wodociągowej

No	Psychrophilic bacteria [cfu/ml]		Mesophilic bacteria [cfu/ml]		Flow cytometry [counts/ml]		
	Agar „A”	Agar „R”	Agar „A”	Agar „R”	LNA*	HNA*	Totality
1	8	16	0	11	2450	19340	21790
2	6	19	1	23	1470	11200	12670
3	2	7	0	9	2890	7800	10690
4	5	14	0	6	3400	11870	15270
5	11	26	2	7	1800	6995	8795

Table 4. The results of bacteriological tests of rainwater

Tabela 4. Wyniki badań baklteriologicznych wody deszczowej

No	Psychrophilic bacteria [cfu/ml]		Mesophilic bacteria [cfu/ml]		Flow cytometry [counts/ml]
	Agar „A”	Agar „R”	Agar „A”	Agar „R”	Totality
1	1800	2000	11800	1500	2874000
2	1470	3400	1900	2400	1983000
3	3100	3400	3000	3800	2344000
4	25000	40000	15000	37000	390000
5	14000	23000	8000	1200	478000
6	3800	4200	3100	3700	2122000
7	22100	36000	15700	27500	1 350000
8	2200	2600	12100	15000	2224000

For flow cytometry, the market offers a wide range of fluorescent dyes targeting specific cell ranges, particles or their biological functions. Because the dyes have been mainly developed for use with cells of mammals, their previous preparation is necessary in order to apply them to microorganisms. Microorganisms and plant organisms have endogenous pigments (chlorophyll, carotenoids), which flow cytometry are detected as autofluorescence-strong red or orange.

Table 5. The results of bacteriological tests of indoor swimming pool water
Tabela 5. Wyniki badań bakteriologicznych wody z krytego basenu kąpielowego

No	Psychrophilic bacteria [cfu/ml]		Mesophilic bacteria [cfu/ml]		Flow cytometry [counts/ml]		
	Agar „A”	Agar „R”	Agar „A”	Agar „R”	LNA*	HNA*	Totality
1	0	95	1	3	930	458	1388
2	1	7	3	24	560	223	783

Autofluorescence is problematic in fluorescent microscopy, because it interferes with the detection of fluorescence signals (> 600 nm). Strong pigment autofluorescence can cause distortion or extinguish if the dye fluoresces in the same frequencies. This affects the quantitative information about the signal from a fluorescent dye. Also autofluorescence can be the cause of such large differences in the size of the microorganism obtained by this method [6]. As of today there are no literature of this type of data. Few sources give similar values for the number of microorganisms as obtained in the studies presented [4,5]. The most reliable results quantities of the microorganism obtained by flow cytometry was obtained for surface water and rainwater. In the case of bacteriological pollution of these waters was a lot higher, and obtained more values close to those obtained for breeding methods. Point to the high sensitivity flow cytometer and detection of fragments of the genetic material of dead micro-organisms in the case of pure waters.

4. Conclusions

- There have been very large differences in the numbers of microorganisms, using traditional breeding methods, and the method of flow cytometry.
- The biggest differences in the numbers of microorganisms in the test methods laid down in the case of observed water, underground water and tap water.
- The smallest differences in the amount of psychrophylic and mesophylic bacteria-marked with the traditional method of breeding, as well as the largest number of microorganisms (i.e., living, dead, including impurities) is marked by flow cytometry, were observed in surface water and rainwater.
- Agar R stimulates the development of a greater number of psychrophylic and mesophylic bacteria than the reference agar-agar A.
- Flow cytometry method is much faster, more accurate (i.e., more sensitive) and also more versatile than traditional breeding methods. However, the applied methodology requires work.
- Traditional breeding methods are time consuming, and greatly underestimate the number of microorganisms contained in the tested water.

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NOWA METODA OCENY JAKOŚCI MIKROBIOLOGICZNEJ WODY

Streszczenie

Ocena jakości mikrobiologicznej wody jest jednym z nadrzędnych zadań inżynierii środowiska. Wiarygodność i dokładność wyników zależny w głównej mierze od systemu próbkowania, a także od metodyki prowadzonych oznaczeń. Zazwyczaj stosowane są tradycyjne metody hodowlane: posiewów płytkowych lub fermentacyjno – probówkowe. W metodach posiewowych zakłada się, że z każdej pojedynczej komórki bakterii przeniesionej na stałe podłoże wyrasta kolonia. Zatem w rzeczywistości wynik badania określa liczbę jednostek zdolnych do tworzenia kolonii, co nie jest równoznaczne z faktyczną ilością żywych komórek bakteryjnych w wodzie. Jeszcze większymi błędami obarczone są wyniki analiz metodami fermentacyjno – probówkowymi, którymi można określić jedynie najbardziej prawdopodobną liczbę bakterii w danej objętości. Dodatkowo metody hodowlane są bardzo czasochłonne. Jest to więc tylko szacowanie ilości mikroorganizmów. Ponadto metody pośrednie są wyjątkowo czasochłonne. Przygotowanie i wykonanie jednej analizy to czas około 3 godzin, a wynik otrzymuje się dopiero po około 1 – 7 dobach. Cytometria przepływowa jest techniką analityczną, pozwalająca na szybki pomiar rozproszonego światła lub sygnałów fluorescencji emitowanych przez odpowiednio naświetlone komórki. Pozwala na jakościową i ilościową ocenę właściwości fizycznych i biologicznych komórek w krótkim czasie. W artykule przedstawiono wyniki badań ilości komórek bakteryjnych w różnych wodach: powierzchniowych, podziemnych, deszczowych i wodociągowych z wykorzystaniem cytometrii przepływowej oraz porównanie tych wartości z wynikami otrzymywanych tradycyjnymi metodami referencyjnymi. Wyniki ilości mikroorganizmów we wszystkich badanych wodach z zastosowaniem cytometrii przepływowej są zdecydowanie wyższe od ilości wykazywanych metodami tradycyjnymi.

Słowa kluczowe: bakteriologia wody, posiewy płytkowe, woda do picia, cytometria przepływowa

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